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TEMPORAL FOOD PREFERENCE AND EFFECTIVENESS OF SELECTED BAIT PRODUCTS AGAINST *PACHYCONDYLA CHINENSIS* (EMERY) (HYMENOPTERA: FORMICIDAE)

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TEMPORAL FOOD PREFERENCE AND EFFECTIVENESS OF SELECTED BAIT PRODUCTS AGAINST *PACHYCONDYLA CHINENSIS* (EMERY) (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Pachycondyla chinensis (Emery), commonly known as the Asian needle ant is a well-established invasive species in urban and woodland areas in South Carolina. Foraging ants are found around or under places such as sidewalks, flowerbeds, mulch, tree bases, stones, and logs where human outdoor activity takes place in urbanized area. It is not an aggressive ant, but it has a powerful sting that causes severe allergic reactions in some people. It also has a negative impact on native ant species in forest environments. Food preference was studied, followed by an evaluation of selected bait products against *P. chinensis*.

Protein, carbohydrate, lipid, and control diets were tested with *P. chinensis* in the laboratory and field. In the laboratory, *P. chinensis* showed no significant preference to any of the food choices. In the field, during the early stages of population growth (late May and early June), the ants showed no significant preference for carbohydrate, lipid, or protein, but visited protein significantly more frequently than plain agar (control), which was the food matrix. When the worker population and swarming activity were higher in late summer (July and August), *P. chinensis* showed a preference for protein over carbohydrate, lipid, and plain agar. These data provided a basis for selection of bait products for efficacy trials.

Seven bait products were chosen to evaluate their effectiveness against *P. chinensis*. In the laboratory, a choice/no choice study was conducted. Advion® fire ant bait, Advance®, and Maxforce® complete achieved 100% mortality in less than one week. Advion® gel reached approximately 90% mortality and was not significantly different

from those reaching 100% mortality after 14 days. When a choice of a natural food source was offered, Optigard® was less effective than in the no choice test, the latter was not significantly different from Advion® gel. Optigard® was not significantly different from Maxforce® quantum in the choice test, while Maxforce® quantum achieved a mortality of 40%. Advion® granule was the least effective bait in both choice and no-choice tests and was not significantly different from control when a choice was available.

Evaluation of bait products in the field was conducted in urban areas where active foraging ants and potential nesting sites were located. Statistically, there were significant differences between treatments in the mean percentage of *P. chinensis* population change in week 1 only. Overall, Advion® gel was the only bait resulting in reduced a field population over 10 weeks. Advion® fire ant bait achieved 70% reduction in the field population during the first three weeks and reduced populations after reapplication in week 7. Advance® was effective in the first seven weeks and had no effect on population reduction in the last three weeks even after reapplication. In the field, Maxforce® complete was not as effective as in the laboratory. The field population increased during most weeks of the study. Optigard® and Maxforce® quantum had similar trends in population change over 10 weeks. The population increased during weeks when there was an increase at control sites, and decreased in weeks when there was a decrease in the *P. chinensis* population at control sites.

DEDICATION

I dedicate this work to my dear grandmother.

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INTRODUCTION

Pachycondyla chinensis (Emery), the Asian needle ant, is an invasive species in the United States which was introduced from Japan (Yashiro et al. 2010). It was first recorded in 1932 in Decatur, GA (Smith 1934). Since then, *P. chinensis* has been recorded in eastern states from Connecticut to the northernmost part of Florida (Paysen 2007, Guénard and Dunn 2010). *Pachycondyla chinensis* is commonly found nesting in rotten logs, beneath the soil surface to depths of 3 to 10 cm, or under objects where moisture is stable (Smith 1934, Paysen 2007). The nests are established around buildings and landscaping in urbanized areas, such as in structural cracks and crevices, flowerbeds, sidewalks, and under garden ornaments and mulch. *Pachycondyla chinensis* stings often cause an allergic response in humans. Stings often occur when ants are trapped between clothing and skin (Nelder et al. 2006). In woodland environments, *P. chinensis* is negatively correlated with the presence of native ant species (Paysen 2007, Guénard and Dunn 2010). However, control recommendations are only speculative.

Objectives and Justification

General chemical control against pest ants can be categorized as direct colony treatment, perimeter treatment, and toxic bait application. Colony treatment requires precise locations of nests or active trails. Because *P. chinensis* builds nests without building a mound, nest entrances are cryptic and multiple, and nest materials often are cluttered. Foraging scouts search for food sources individually. Ants are not inclined to invade buildings, but may be brought in accidently by humans or fly into structures during the swarmer stage. Because of this behavior, exclusion of *P. chinensis* using a perimeter treatment is not applicable. Colony treatment and perimeter treatment are inefficient, costly, and impractical against *P. chinensis*.

Baiting is an important control method for pest ants with many advantages. One advantage is that bait is easy to use and requires only a small amount of toxicant. Another advantage is that bating specifically targets attracted species and allows for distribution of chemical to all members of the colony. Unlike insecticidal sprays, baiting is less likely to require knowledge of the precise nest location. In studies with other species, when used with a proper combination of non-chemical strategies, baiting can provide sufficient long-term control (Lucas and Invest 1993, Silverman and Rouslton 2001, Stanley 2004). The goals of this research were to 1) determine the temporal food preference of *P. chinensis* in both the laboratory and an urbanized field environment and 2) test the efficacy in the laboratory and field of bait products which were selected based on the results of the laboratory study.

CHAPTER ONE

LITERATURE REVIEW

Pachycondyla chinensis (Emery) (Formicidae: Ponerinae) is an invasive species in the United States. It was first recorded in 1932 at Decatur, GA and also was documented from a number of localities in North Carolina and Virginia (Smith 1934). Its current distribution is reported in the eastern states from Connecticut to the northernmost part of Florida (Guénard and Dunn 2010). This species was intercepted in Hamburg, Germany, in a shipment of plants from Japan, and presumably it reached North America by a similar route (Smith 1934). The molecular analysis based on mitochondrial sequences of the COI region showed no difference between *P. chinensis* from the United States and those from the temperate areas in Japan (Yashiro et al. 2010). Both documentation and phylogenetic analysis suggest that *P. chinensis* has been introduced into the United States from Japan.

Pachycondyla is a large and diverse genus consisting of 252 recognized species found in tropical and subtropical regions of the world (Bolton 1995, Bolton 2012 updated). *Pachycondyla chinensis* is native to Japan and parts of China (Creighton 1950), and the type locations are there as well (Bolton 1995). It is now widely distributed in more regions, including New Zealand, the United States, Vietnam, South Korea, Taiwan, Guam, India, Indonesia, Myanmar, Nepal, Papua New Guinea, Philippines, Solomon Islands, Sri Lanka, and Thailand (Xu 1994, Bolton 1995, Nelder et al. 2006).

In Japan, it is considered there are two species in the *P. chinensis* complex distinguished by analysis of mitochondrial sequences of the COI region and key characters of the worker and male (Yashiro et al. 2010). One species is *P. chinensis* which was introduced to the United States, and the other is *P. nakasujii* (Yashiro). They are widely sympatrically distributed and abundant (Yashiro et al. 2010).

Biology and natural history

Pachycondyla chinensis was originally described by Smith as a junior primary homonym of *Ponera solitaria* (Smith) in 1860 and junior synonym in 1874 (Bolton 1995, Bolton 2012). The accepted description was published as *Ponera nigrita* subsp. *chinensis* by Carlo Emery in 1895, *Annali del Museo Civico di Storia Naturale di Genova* (Emery 1895, Bolton 1995). It was named *Euponera (Brachyponera*) *chinensis* by Emery due to generic combination in *Euponera* in 1909, replaced with the name *Brachyponera chinensis* by Brown in 1958, and combined into the genus *Pachycondyla* by Bolton in 1995 (Bolton 1995, Brown 1958).

Table 1.1 Systematic summary of *Pachycondyla chinensis* (Bolton 1995, Paysen 2007)

Name	Date	Author	Details
Ponera solitaria	1874	Smith F.	Original description of worker. Hiogo, Japan.
Ponera nigrita subsp. *chinensis*	1895	Emery	Description of worker. Junior synonym. China.
Euponera (*Brachyponera*) *chinensis*	1909	Emery	Generic combination.
Brachyponera chinensis	1958	Brown	Junior synonym of *B. solitaria* Junior homonym of *B. solitaria*
Pachycondyla chinensis	1995	Bolton	Generic combination

The worker of *P. chinensis* is black with reddish brown maxillae, flagella, legs and pygidium. Antennal scapes are robust reaching beyond the posterior corners of the head. Compound eyes are rather large. There are six or seven teeth on the interior edge of maxilla. The occiput edge is straight. Pronotum appears convex. Mesonotum is surrounded by the circular suture. They have one node which is unusually large, erect, and convex anteriorly and flattened posteriorly (Smith 1934). There is a high density of setae on the head and body compared to a low density on the prothorax, node and abdomen (Smith 1934, Xu 1994). The gaster occupies the greater portion of the surface when viewing dorsally (Smith 1934).

The *P. chinensis* queen is the same color as the worker, but with a longer body length and a set of wings which are lost before oviposition begins (Tebeau 2009). The male also bears a set of wings, is smaller in body size than the female, and a lighter pale brown color than the female (Smith 1934, Yashiro et al. 2010).

The larvae of *P. chinensis* go through four instars which are differentiated by head width (Wheeler and Wheeler1986). The body bears numerous tubercles and one or two intersegmental mid-dorsal discoids or doorknobs which are used to adhere to the surface of nesting material (Wheeler and Wheeler1986, Tebeau 2009). They share a unique morphological modification of a food platter with larvae of the ponerine ants in the genera *Odontomachus* and *Pachycondyla* (Hölldobler and Wilson 1990). The food platter is a posterior region on the ventral side of the body, wide and flattened to hold the food while feeding (Hölldobler and Wilson 1990, Tebeau 2009). The appearance of varied sizes of larvae adhering beneath an objects moist surface when searching for *P. chinensis* nests is very distinct in the field. The pupa is about 4.0 mm, brown, and rice-like (Tebeau 2009). A mass of pupae found under nesting objects also was distinct in urbanized area.

In western Japan, Gotoh and Ito (2008) studied seasonal cyclical changes in colony structure of a species thought at the time to be *P. chinensis*. It was later determined by DNA analysis to be *P. nakasujii* (Yashiro et al. 2010). *Pachycondyla nakasujii* was found nesting in dead branches, bamboo sheathes, and a small space between fallen leaves on the ground in the forest floor (Gotoh and Ito 2008). In Japan, *Pachycondyla nakasujii* and *P. chinensis* are sympatrically distributed in temperate zone, but *P. nakasujii* is rare in dry and disturbed areas while *P. chinensis* remains common throughout Japan (Yashiro et al. 2010). There is little known about the seasonal colony structural composition of *P. chinensis*.

Pachycondyla chinensis was described as being seen everywhere on rice paddy dikes and in farm compounds in China (Brown 1958). It forms small colonies in rotten logs, or in the soil beneath stones, logs, debris, galleries associated with structural objects including logs, rocks, subterranean tree roots, and bricks, boards, or other human-made debris (Smith 1934, Smith 1947). Paysen (2007) found *P. chinensis* nests established around buildings and landscaping in urbanized areas, on the forest edges, and interior forests in South Carolina. Nests are shallow, beneath the soil surface to depths of 3 to 10 cm, or located above the surface in decomposing logs, but always where moisture is stable (Paysen 2007).

Pachycondyla chinensis opportunistically feeds on live or dead insects, fish scraps, and decaying fruit. Ants forage on the ground in open areas and on tree trunks in forested areas (Brown 1958). They prey on small live arthropods. In a study conducted in North Carolina, prey for *P. chinensis* included Curculionidae (Coleoptera) adults, Pyralidae (Lepidoptera) larvae, Mycetophilidae (Diptera) larvae, Diplopoda, Machilidae (Archaeognatha), *Reticulitermes virginicus* (Banks)(Isoptera) workers, Elateridae (Coleoptera) larvae, Trogossitidae (Coleoptera) adults, Chilopoda, Parcoblatta spp. (Dictyoptera) larvae, and Collembola (Bednar and Silverman (2011). They showed a preference for *R. virginicus* among these potential prey items (Bednar and Silverman 2011).

Pachycondyla chinensis perform individual foraging during which they forage without systematic cooperation or communication in finding, capturing, or transporting food. They also engage in tandem carrying recruitment, foraging with a worker carried directly to food source by a scout (Beckers et al. 1989).While tandem carrying behavior has been indentified in *P. chinensis*, their recruitment is not known to involve pheromones, and does not result in trails of workers going to a food source as with Argentine or odorous house ants (Beckers et al. 1989). The occurrence of tandem carrying in foraging is dependent on the food source (Guénard and Silverman 2011).

<u>Ecological impact</u>

Pachycondyla chinensis has demonstrated a strong negative impact on native ant species abundance and diversity in hardwood forests (Paysen 2007, Guénard and Dunn 2010). The threat to ecological systems and diversity was predicted and shown by Paysen (2007), and Guénard and Dunn (2010) studied the relationship between *P. chinensis* and native ant species in mature hardwood forests. Both studies reported the presence of *P. chinensis* was negatively correlated with composition, abundance and diversity of native ant species. Further *P. chinensis* was demonstrated to be associated with the disruption of ant-plant seed dispersal mutualism, and potentially responsible for reduced abundance of ant-dispersed plants (Rodriguez-Cabal et al. 2011).

Pachycondyla chinensis was described as a termite feeder that occupies subterranean termite galleries, preying on *R. virginicus* (Teranishi 1929, Paysen 2007, Bednar and Silverman 2011). Besides the relationship with native ant species, Bednar and Silverman (2011) identified *P. chinensis* using termites as a food source, particularly *R. virginicus*, as a springboard for their success as an invasive species. They speculated this may deplete native subterranean termite populations and negatively impact long-term ecosystem processes (Bednar and Silverman 2011).

Medical importance

Pachycondyla chinensis stings often cause an allergic response in humans. The stings are described as itchy, causing intense pain that fades away and returns in several hours, and the pain is not always in the area of the original sting site (Nelder et al. 2006). Pain from stings also may be described as feeling repeated stings at the original site lasting for several minutes (Mo personal experience). Symptoms include generalized urticaria, respiratory distress, wheezing, and hypotension with or without loss of consciousness (Kim et al. 2001). Based on a sample from the patient's home, Leath et al. (2006) reported the first case of anaphylaxis to *P. chinensis* in the USA using immunoblot analysis. Stings often occur when the ants are trapped between clothing and skin (Nelder et al. 2006).

Nelder et al. (2006) conducted a survey of people who experienced *P. chinensis* stings at the Greenville zoo. The first time *P. chinensis* was reported as a problem at the zoo was during the building of a new exhibit in 1997. Zoo personnel considered *P. chinensis* a pestiferous insect.

Ant control

General ant control in urban landscapes is handled using techniques and practices in keeping with integrated pest management (IPM), but often include the application of residual insecticides and toxic baits (Silverman and Brightwell 2008, Mallis 2011). The concept of IPM is widely used in many areas of insect control in urban and agricultural settings. In the managed urban area, IPM programs mainly involve altering the nesting habitat and reducing the food supply of targeted species, before the application of a liquid or bait toxicant (Silverman and Brightwell 2008). Knowledge of ecology and biology of targeted species is critical for success.

There is wide diversity in chemical management against pest ants which varies with the ecology and biology of targeted species. Mallis (2011) summarized these chemical treatments into residual insecticide applications which are applied directly to the nest or perimeter around a structure, and toxic bait application. Some of the applications include individual mound treatment, perimeter treatment, and spot treatment (Lofgren et al. 1975, Oi et al. 1996, Rust et al. 1996, Klotz et al. 2002, Scharf et al. 2004, Klotz et al. 2007).

Individual mound treatment can be used for ants nesting within identifiable mounds and is varied with the habitat of pest species. Chemical formulations commonly used are dust, liquid, granule, or aerosol (Mallis, 2011). The behavior and colony life history information of targeted species is important for making targeted applications.

Mound drenches were often used in red imported fire ant management with a liquid concentrate diluted in water (Morrill 1976, Drees 2002). Toxic bait was also used in individual mound treatments (Lofgren et al. 1975, Weeks et al. 2004).

Spot treatments with contact insecticides are usually applied when active ant trails or the nest is located (Klotz et al. 2007). Ant activity is reduced immediately by individual mound treatment and spot treatment if proper insecticide and delivery are chosen. The precise identifications of nest locations and active areas are critical to successful individual mound and spot treatments.

Perimeter treatments can be applied around a foundation wall or food source to prevent ants from invading a structure (Lofgren et al. 1975, Oi et al. 1996, Rust et al. 1996, Klotz et al. 2002, Scharf et al. 2004). Insecticides which have activity as a contact, repellent or non-repellent, or residual toxicant, act as a barrier around target areas. It can be used to immediately eliminate an infestation, or for long-term prevention depending on the properties of the insecticide. Barrier spray was often used in managing Argentine (*Linepithema humile* Mayr), odorous house (*Tapinoma sessile* Say), and carpenter ants (*Camponotus spp.*) (Rust et al. 1996, Tripp et al. 2000, Klotz et al. 2002, Scharf et al. 2004, Klotz et al. 2007). However, factors which reduce the performance of residual insecticide efficacy, such as heavy irrigation, dense ground cover, and direct sunlight, may create a gap around the barrier and result in immigration of ants from untreated areas (Rust et al. 1996, Vega and Rust 2003). Insecticide treated mulch was tested as a barrier against Argentine ants, but the effectiveness was limited (Meissner and Silverman 2001, Meissner and Silverman 2003).

Toxic bait is commonly used in ant control. It is easy to apply, less likely to be impacted by surface conditions or precise nest location than insecticidal spray, provides sufficient long-term control with a proper combination of treatments, is distributed to all members of the colony, specifically targets attracted species, and requires only a small amount of toxicant (Lucas and Invest 1993, Silverman and Rouslton 2001, Stanley 2004, Warner et al. 2008).

Bait consists of four components: an attractant, a palatable carrier, a toxicant, and other materials added for reasons of formulation (Klotz et al. 1997). The attractants in ant baits are food or a pheromone which encourage ants to bring the bait back to the nest. An active ingredient with delayed toxic effect of <15% mortality at 24h or > 89% mortality at the end of test, was considered preferable as it allowed for distribution to the remainder of the colony in controlling of the red imported fire ant (*Solenopsis invicta* Buren) (Stringer et al. 1964). A bait product with a palatable attractant is important, and it requires understanding the target species biology including food preference, foraging behavior, and colony life history.

CHAPTER TWO

TEMPORAL FOOD PREFERENCE IN PACHYCONDYLA CHINENSIS (EMERY) (HYMENOPTREA: FORMICIDAE)

Introduction

In 1932, *Pachycondyla chinensis* (Emery) was first recorded in Decatur, Georgia (Smith 1934). The range has extended from the first reports in Georgia, North Carolina, and Virginia to most eastern states (Smith 1934, MacGown 2009, Guénard and Dunn 2010). *Pachycondyla chinensis* has demonstrated a strong negative impact on native ant species abundance and diversity in hardwood forests (Paysen 2007, Guénard and Dunn 2010). Native ant species, which are keystone species for seed dispersal mutualisms in hardwood forests, were significantly reduced in environments where *P. chinensis* occurred (Paysen 2007, Rodriguez-Cabal et al. 2011). *Pachycondyla chinensis* was described as a termite feeder and occupies subterranean termite galleries, preying on *Reticulitermes virginicus* (Banks) (Teranishi 1929, Paysen 2007, Bednar and Silverman 2011). The number of sting cases by *P. chinensis* (unpublished data) and public awareness of this species as a health threat has increased since it was first documented in the US (Nelder et al. 2006, Jackson 2012). The negative effects of *P. chinensis* are exhibited in both its long-term ecological impact and medical importance.

Pachycondyla chinensis is polydomous. Nests that are no more than 25 cm apart are considered to be part of the same colony (Paysen 2007). This species nests in logs and beneath natural and human-made objects. Nests are situated in protected soil to a depth of 3-10 cm. Nest entrances are hidden and cryptic. Typically, ant control includes applications of residual insecticides and baits (Mallis 2011). Application of residual insecticides is generally categorized into colony treatment, which is applied directly to the nest, and perimeter treatment around a building (Mallis 2011). Targeting all nests with residual insecticide for *P. chinensis* is difficult and inefficient, considering the nesting habits of this species. Using baits to control *P. chinensis* may prove to be an important strategy if an effective product can be identified.

Little is known about the food preferences of *P. chinensis*. Food preference is critical in attracting foraging ants when designing a bait matrix. *Pachycondyla chinensis* performs individual foraging or tandem carrying recruitment depending on the food source (Guénard and Silverman 2011). Without mass recruitment by trailing, a food source must be palatable and attractive. Three major food types (proteins, carbohydrates, and lipids) are used in bait matrices (Barbani 2003, Cook et al. 2011). These three food types which are found in different bait products were previously tested in food preference studies with the odorous house ant (*Tapinoma sessile* Say), the southern fire ant (*Solenopsis xyloni* McCook), the Argentine ant, (*L. humile* Mayr), the California harvester ant (*Pogonomyrmex californicus* Buckley), the red imported fire ant (*Solenopsis invicta* Buren), and the crazy ant (*Paratrechina longicornis* Latreille). The ingredients included chicken egg, casein, anchovy and tuna for proteins; honey and sucrose for carbohydrates; and peanut oil, vegetable oil and extra virgin olive oil for lipids (Hooper and Rust 1997, Hooper-Bui et al. 2002, Barbani 2003, Weeks et al. 2004, Stanley and Robinson 2007). Based on the nutritional facts for these foods, we determined that canned tuna, sucrose, and vegetable oil were less adulterated than other possible choices (http://www.nutrientfacts.com 2009 and http://nutritiondata.self.com 2012) (Table 2.1). They were also convenient to purchase inexpensively and thus, were chosen for this study.

Table 2.1 Nutritional facts for food used in previous studies of food preference for ants (obtained from http://www.nutrientfacts.com 2009 and http://nutritiondata.self.com in 2012)

Food	Carbohydrate	Protein	Fat
Tuna canned in water	0.00%	24.45%	0.00%
Chicken egg	0.00%	12.00%	10.00%
Casein	9.00%	72.72%	3.00%
Anchovy canned in oil	0.00%	28.88%	11.11%
Honey	67.38%	0.00%	0.00%
Sucrose	100.00%	0.00%	0.00%
Peanut oil	0.00%	0.00%	100.00%
Vegetable oil	0.00%	0.00%	100.00%
Extra virgin olive oil	0.00%	0.00%	100.00%

In nature, factors such as colony size, foraging surface conditions (including vegetation, temperature, relative humidity, and predators), food availability, competition with other arthropods, reproductive status, and fitness are varied (Schoener 1971, Bailey and Polis 1987, Hölldobler and Wilson 1990, Kay 2002). For this reason, food preference studies were conducted in the laboratory and the field to determine temporal nutritional preferences of *P. chinensis.*

We hypothesized that (1) *Pachycondyla chinensis* prefers protein- and carbohydrate- to lipid-based foods, and that protein will be most attractive, followed by carbohydrates, and (2) foraging to a specific food source will be significantly different based on the colony structure and life stages present as indicated by the season.

Material and Method

1. Food preference study in the laboratory

Colony Collection

Nests were excavated from sites in Clemson, Pendleton, and Seneca (South Carolina, USA) by removing soil and material surrounding the nest with a shovel. Nests collected were more than 20 m apart. Individual nests were placed separately in 18.9 liter plastic buckets and returned to the laboratory (Clemson University Urban Entomology Laboratory, Clemson, South Carolina, USA). Each nest was considered a separate colony.

Colonies were kept in a plastic container with a diet of termite workers (*Reticulitermes* spp.) or late stage mealworm larvae (*Tenebrio molitor* L.) in the laboratory and held at 22 ± 3ºC, RH 65%. Water was provided by placing a water-soaked sponge plugged into a glass test tube (25 cm long, 25 mm in diameter) in the container. Both food and water were provided *ad libitum*.

Ants were separated from the debris and soil with a sieve kit (wire mesh sizes of 40 mm, 2 mm, 250 μm and 63 μm) 24-48 hours before conducting the laboratory studies. The largest sieve was placed on top and smallest on the bottom. Coarse soil, debris, and stones did not pass through the first sieve. Ants, small debris, and soil passed through to the bottom container. Ants were collected from the bottom sieve along with fine soil material, and kept in a small container. Water was provided by misting ants daily.

Water-packed tuna (StarKist Co. Pittsburgh, PA) was used as the protein source. Tuna was homogenized with a mortar and pestle and distilled water was added and thoroughly mixed. Moist agar (2% agar, Carolina Biological Supply Company, Burlington, NC) was melted in a microwave oven and kept in a hot bath at approximately 80ºC. Homogenized tuna was mixed with dissolved agar in a beaker, warmed on a hotplate at approximately 60ºC. The carbohydrate source was prepared with sucrose (Carolina Biological Supply Company, Burlington, NC) dissolved in distilled water. The sucrose solution was mixed with agar and prepared as previously described. Vegetable oil was used as the lipid source (Crisco, the J. M. Smucker Company, Orrville, OH). Oil was distributed in warm, distilled water by stirring, and mixed with agar and prepared as previously described.

All materials were measured by weight (Table 2.2). To each food, 3.50 g distilled water and 3.50 g agar was added. Once prepared, the mixture was transferred into a canning jar and placed into an ice bath. The mixture was continually shaken while in the ice bath. After the mixture gelled, it was stored in the refrigerator.

Table 2.2 Ingredients used in each food type (by weight) for the study of food preference of *Pachycondyla chinensis* (Emery)

	Concentration (%)	Sucrose (g)	Canned tuna (g)	Vegetable oil (g)
Carbohydrate	10.00	0.56	0.00	0.00
	20.00	3.50	0.00	0.00
	30.00	1.50	0.00	0.00
Protein	3.00	0.00	0.43	0.00
	5.00	0.00	0.76	0.00
	10.00	0.00	1.79	0.00
Lipid	1.00	0.00	0.00	0.05
	3.00	0.00	0.00	0.15
	5.00	0.00	0.00	0.26
Plain agar[1]	0.00	0.00	0.00	0.00

[1]The composition of plain agar is agar only with no other food added.

Experimental Design

The specific food source is one of the inert ingredients in bait that plays a role in attracting foraging ants. The formulation and type of food are considered when designing a bait matrix. The type of food that represents energy intake, time spent searching for food, handling food, and returning it to the nest is also important in optimal foraging theory (Schoener 1971, Pyke 1984, Bailey and Polis 1987). Time spent searching for food and returning it to the nest can be controlled by the placement of food. Using foods of similar texture in a test imparts similar handling properties for the ant. Food was made into a gel that was easily cut and carried by *P. chinensis* workers, and was firm enough to retain its integrity. Particle size and placement of food were also controlled.

The food preference study was performed in the laboratory where a) colony size was fixed by selecting the same number of ants for each treatment, b) colonies were collected during the same season and maintained in the laboratory at a constant temperature and relative humidity, c) the experiment was conducted under the same temperature and relative humidity, and d) foraging area was fixed by using the same study arena for all treatments. Controlling factors affecting foraging behavior and food selection allowed for comparison of food preference.

Prior to conducting the laboratory study, ants were separated from the colony and held for three days without food. Twenty worker ants were aspirated from the starved cohort (~60-200) and were placed in a plastic Petri dish (15 cm in diameter) as an experimental unit for the test. A water-soaked cotton ball was put in the center of the dish with a plastic cover to prevent rapid desiccation. Food was placed on half a plastic hexagonal weigh boat (side length of top 2.5 cm, bottom 1.5 cm) and positioned next to the Petri dish wall.

A preliminary test was conducted to determine an attractive concentration for each food investigated in the study. Carbohydrate concentrations of 0, 10, 20, and 30%, protein concentrations of 0, 3, 5, and10%, and lipid concentrations of 0, 1, 3, and 5% were tested. For all foods, 0% concentration was the agar matrix only. Four concentrations of each type of food were put in half a weigh boat and placed in the Petri dish equidistant from the center in four directions. The number of ants visiting each food type was counted every 30 minutes for two hours. The most visited concentrations of each type of food were chosen. Each colony was assigned to each food type at least once and was used in more than one replicate if the number of cohorts was high. Each Petri dish setup (experimental unit) was a replicate. A total of ten replicates were conducted with seven colonies.

Based on preliminary data, the preference study was conducted by providing these three food types at concentrations of 20% carbohydrate, 3% lipid, and 5% protein to 20 worker ants as previously described. Again, the number of ants visiting each food was recorded every 30 minutes for two hours. Three treatment boats were randomly arranged in the Petri dish equidistant apart. Four replicates were conducted with four colonies used in the food preference test.

Data Analysis

Both the preliminary test and preference study in the laboratory were conducted as a complete block design. A colony was considered a block effect. The numbers of replicates conducted in each block varied depending on the colony size. The difference in the average number of ants visiting each concentration of a food type in the preliminary study or visiting different food types in the preference study was compared using analysis of variance, followed by the Fisher's least significant difference test (PROC GLM, SAS institution 9.3 2011).

2. Food preference study in Field

Study sites

In preliminary trials, we found that there was more competition for food from other species of insects in wooded areas than in urban areas. Consequently, sites were selected in urban locations for the field study in Clemson and the surrounding area (Pickens Co. and Anderson Co., South Carolina, USA). Each site had active *P. chinensis* foragers, and the nest site could be located and entrances found. If multiple entrances were found, the most active entrances were marked.

Food was prepared as in the laboratory food preference study. Parchment paper (Reynolds, Consumer Products Inc., Richmond, VA) was cut into 1 x 2 cm pieces and was used for food placement in the field. This paper was waterproof and discouraged ants from crawling under the paper for moisture. The paper was placed to ensure ants were able to walk on the paper during the trials.

The three types of food and a control were put separately onto the parchment paper. The control was a mixture of agar and water only. Four pieces of parchment paper, each with a different food, were randomly placed approximately within 20 cm of the nest entrance of a colony (Takimoto 1988). The test was conducted twice at each site by placing a new set of food on another side of the nest. The second placement was approximately 1 m away from the first placement, and was also within 20 cm of an entrance. Each placement was considered one replicate.

The number of ants visiting each food type was recorded in 30 minutes after the first ant was observed at any of the food placements. A visiting ant was defined as one moving onto the paper regardless of whether or not it removed food. In the early spring, late fall, and winter, actively foraging *P. chinensis* are scarce and ground surface nesting sites are not abundant. The food preference study in the field was conducted in two periods, first as the population began increasing, and second, at the peak of population. Period 1 was conducted with four replicates in late May and early June in two locations. Period 2 was conducted with sixteen replicates in late July and early Aug in three locations. The numbers of replicates were different between those two periods because the method of counting ant visits in Period 1was altered when data were insufficient to reflect the true foraging pattern, and there were fewer available foraging sites during period 1 than period 2. Data were originally collected based on counts made for five minutes in every 30 minutes for two hours, while the final ant foraging counts were made continuously for 30 minutes.

Data Analysis

The foraging activity for each period was evaluated by Analysis of Variance. A mixed model was used in the SAS procedure (PROC MIXED, SAS institution 9.3 2011). Fixed effect was food type. Random effects in the model included location which varied with different environmental factors, site where the colony nested, and entrance where ants were observed. Site was nested within location and entrance was nested within site. A Tukey test was used for the pairwise comparison when there were significant differences in average number of ants visiting each type of food in 30 minutes.

Voucher specimens were deposited in the Clemson University Arthropod Collection at Clemson University.

Results

1. Food preference test in laboratory

In the preliminary test to determine an effective concentration of carbohydrate, the mean number of ants visiting each concentration did not differ significantly ($F=0.83$; $df=3, 30$; $P>0.05$). Carbohydrate at a concentration of 20%, used in the study that followed also was reported as acceptable in previous studies of other ant species (Barbani 2003, Nelson and Daane 2007). The mean numbers of ants visiting each concentration of lipid were significantly different ($F=6.01$; $df=3, 30$; $P=0.003$). Comparison of each concentration, using a pairwise t-test, showed that a significantly higher number of ants visited the 3% concentration. The mean number of ants visiting each concentration of protein also was significantly different ($F=3.62$; $df=3, 30$; $P=0.024$). A pairwise t-test showed that significantly higher numbers of ants visited protein at concentrations of 5 and 10% compared with plain agar (0%) (Table 2.3). Therefore, concentrations of carbohydrate at 20%, lipid at 3%, and protein at 5% were used for laboratory and field studies.

Table 2.3 Mean number of *Pachycondyla chinensis* (Emery) visiting each concentration of three food types in a preliminary laboratory study (1) to determine optimal food content for each food type used in the food preference study (2).

Food type	Concentration	1[1]	2[2]
		Mean ± SD	Mean ± SD
Carbohydrate (%)	0	3.600 ± 5.680a	
	10	5.200 ± 5.903a	
	20	5.900 ± 6.385a	16.250 ± 16.540a
	30	5.200 ± 7.800a	
Lipid (%)	0	2.000 ± 2.108b	
	1	2.800 ± 2.616b	
	3	8.100 ± 6.506a	5.750 ± 2.630a
	5	2.400 ±2.836b	
Protein (%)	0	1.500 ± 1.434b	
	3	3.600 ± 2.836ab	
	5	5.700 ± 5.538a	6.500 ± 2.517a
	10	5.600 ± 6.398a	

[1]is the mean ± SD of visiting ants in the preliminary concentration study. Means followed by the same letter, within the same food type category, and within the same column, were not significantly different (P>0.05). [2]is the mean ± SD of visiting ants in the laboratory food preference study. Means followed by the same letter, within the same column, were not significantly different (Fisher's least significant difference test; P>0.05).

In the laboratory food-preference test, carbohydrate, lipid, and protein showed no significant differences in mean numbers of ants visiting each food type in two hours ($F=1.74$; $df=2, 6$; $P>0.05$).

2. Food preference test in field

The population of *P. chinensis* in each colony in the field varied throughout the study. We computed the number of ants visiting each type of food, including plain agar, carbohydrate, lipid and protein in the field as a proportion separately at each site in the analysis.

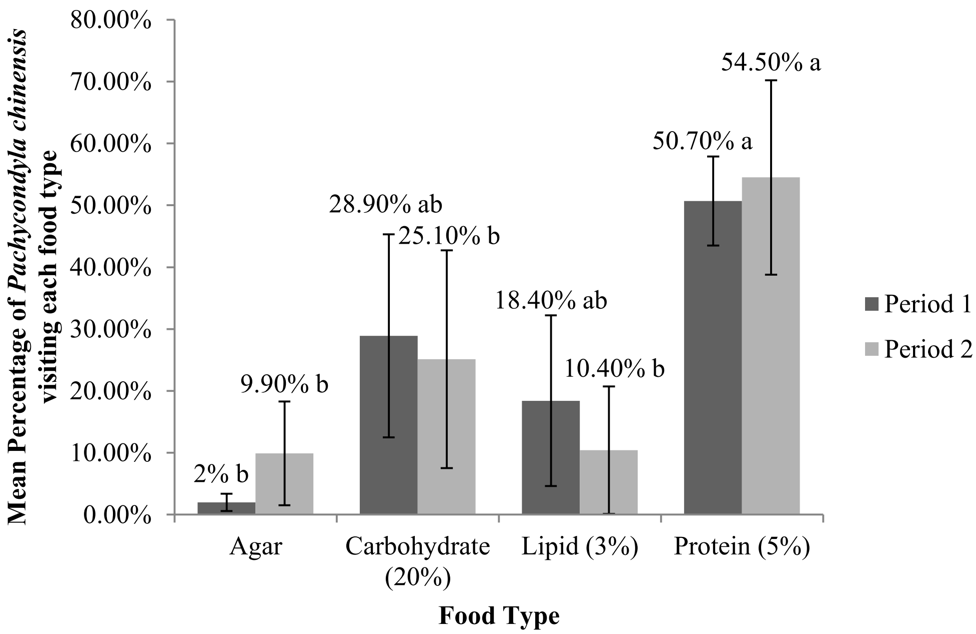

Figure 2.1 Mean (± SE) percentage of *Pachycondyla chinensis* (Emery) visiting each food in the field. Means labeled with same letter within each period were not significantly different.

In late May and early June, Period 1, the mean percentages of ants visiting agar, carbohydrate, lipid, and protein in 30 minutes were significantly different ($F=13$; $df=3, 3$; $P=0.0004$). Comparison of these four types of food showed the percentage of ants visiting protein was not significantly greater than the percent visiting carbohydrate or lipid (Fig. 2.1). The mean percentage of ants visiting carbohydrate and lipid was not significantly greater than plain agar. However, ants visited protein significantly greater than plain agar. The percentage of ants visiting protein was 50.7%.

In late July and early August, Period 2, there were significant differences in the mean percentage of ants visiting each type of food ($F=37.99$; $df=3, 6$; $P<0.0001$). The percentage of ants visiting protein was 54.5% which was significantly higher than the other two food types and control (Fig. 2.1). No significant differences were found in the percentage of ants visiting carbohydrate, lipid, and plain agar.

The mean percentage of ants visiting each food type showed similar trends, with protein the highest, carbohydrate the second, lipid the third, and agar the lowest in both periods. However, the percentage of ants visiting protein was the only one significantly greater than plain agar.

Discussion

1. Food preference test in laboratory

In the preliminary laboratory test to determine effective food concentrations, *Pachycondyla chinensis* had choices between plain agar and different concentrations of each food type in each test Petri dish. Ants visited all food types provided during the study, and showed no significant difference in the average numbers visiting each food type. In contrast to the initial hypothesis, *P. chinensis* did not show significant preference over different food types using collected colonies in the laboratory study.

The food preference of ants is predicted to vary with colony size, environmental factors influencing foraging, food availability and quality, soil surface conditions, reproductive status, and colony fitness (Bernstein 1975, Breed et al. 1987, Beckers et al. 1989). These factors may be reflected in the choices made by foraging ants. Ants in the colonies collected for laboratory studies were separated from field nesting material by multiple-screen-sizes of sieves. Ants collected on the bottom sieve were adult stage. The majority of larval stages were lost in the separation. The laboratory habituation time was not long enough for the disrupted colonies to reestablish the population structure they had in the field and may influence food preference and metabolic needs.

Varied seasonal food collection behaviors and nutrient regulation strategies may be an adaptation that allows long-lived animals to meet current and future nutrient demands when nutrient-rich foods are abundant, and to conserve energy and be metabolically more efficient when nutritionally balanced foods are less abundant (Cook et al. 2011). The composition of adult reproductives was demonstrated to influence the foraging decision directly in *Pheidole ceres* (Wheeler) in a laboratory study (Judd 2005). Colonies in the laboratory studies were mainly composed of worker ants and maintained under identical photoperiod, temperature, and relative humidity; therefore, the food preference study results might not reflect the nutritional requirements in the field, but rather, only the current short-term worker ant energy intake and immediate colony metabolic nutrient demands (Portha et al. 2002). Lipid and protein can also be converted to carbohydrate as fuel for energy metabolism with different synthesis cost (Chapman 1998, Barbehenn et al. 1999).

Multiple behaviors were observed (e. g., individual food carrying, scouting, liquid removal on site, covering food sources with nesting materials, and recruiting by tandem carrying; (Guénard and Silverman 2011)) when the number of *P. chinensis* visiting each food was recorded. Each mixed food type was moist and unlimited in the study. The observation of visiting ants in the laboratory study without larval and reproductive stages might not show food preferences of *P. chinensis,* but rather acceptance of different food types. In the laboratory preliminary and food preference studies, *P. chinensis* visited plain agar, carbohydrate (sucrose), lipid (vegetable oil), and protein (canned tuna) indicating no clear preference.

2. Food preference test field

Food preference studies have been conducted with several ant species. The Argentine ant (*Linepithema humile* Mayr) and odorous house ant (*Tapinoma sessile* Say) both showed preference for sucrose water over a protein food (Baker et al.1985, Barbani 2003). The odorous house ant preference for carbohydrate did not change over the season. The black carpenter ant (*Camponotus pennsylvanicus* DeDeer) also fed more on carbohydrate than protein, and negligibly on lipid. However, the collection of protein was higher during July and Sept when more mature larvae were present (Cannon and Fell 2002). Food preference of *Pheidole ceres* (Wheeler) varied over three different developmental phase. It showed no significant preference between carbohydrate and protein during spring when a large number of larvae were present in the colonies. A significant preference for carbohydrate was exhibited during the reproductive phase in summer and fall (Judd 2005). The red imported fire ant, *Solenopsis invicta* Buren, was recruited more often to carbohydrate in the colder part of the season, and protein in the warmer part of the warm season (Stein et al. 1990). Protein and carbohydrate were more preferred over lipid in these ants; and the food preference of *P. chinensis* in this study also showed similar preference for protein and carbohydrate food. However, the significant shift in preference for protein was detected during the peak population period in late summer which is different from most ants previously studied.

In the field preference study, multiple behaviors were observed as in the laboratory test. In both periods, ants visited all four types of food. Specific action taken by each visiting ant was not recorded, but we did observe that consuming liquid from the food matrix on site slowed foraging activity, which resulted in less food transport than other feeding behaviors. The higher percentage of visiting ants should reflect higher foraging activity and more frequent food transportation.

Little is known about the seasonal development of *P. chinensis*. In South Carolina, *P. chinensis* worker activity has been consistently detected in pitfall traps in late March, with worker numbers steeply increasing in late April and gradually in May and June (Zungoli and Benson 2008). The worker ants captured in pitfall traps increased in number in July and Aug and peaked in late July/early Aug (Zungoli and Benson 2008). In the food preference study, late May and early June (Period 1) was considered the beginning of the population increase, and late July and early August (Period 2) was considered the peak of population activity with active swarmers and increased worker numbers.

In Period 1, *P. chinensis* showed a preference for protein significantly over plain agar. However, the number of visits to carbohydrate and lipid were not significantly different than to protein, but both of those food types were also not significantly different than agar. Significant differences may have been detected with a higher number of replicates. At the peak population, Period 2, *P. chinensis* visited protein significantly more than the other three foods. *Pachycondyla chinensis* visited the protein, carbohydrate, and lipid sources early in the season when the population was growing. And at the peak population, the foragers collected significantly higher amounts of protein over carbohydrates and lipids. In both periods, there was a similar trend in the preferred food. Protein was most visited. It was followed by carbohydrate, lipid and the food matrix (plain agar). The results in Period 1 were not significantly sufficient to conclude a food preference in *P. chinensis* as stated in the hypothesis. However, protein was significantly preferred in Period 2, followed by carbohydrate and lipid as hypothesized.

Pachycondyla chinensis alates were captured starting in late May, and peak activity as measured by NJ light traps occurred in late July/early Aug (Zungoli and Benson 2008). The cycle of colony structure is not well known. Pitfall traps capture only ants on the ground and light traps capture only alates when swarming activity begins. The development and composition of colony members are unclear. High reproductive activity should occur during or after the peak of alates. Protein-rich food is required for successful reproduction. At the peak of swarming activity, which was late July and early August, *P. chinensis* collected protein in a significantly higher amount than carbohydrate and lipid.

Macronutrient (carbohydrate, protein, and lipid) requirements in insects include amino acid for development which is attained from protein and generates fuel for energy metabolism (Barbehenn et al. 1999). Since both lipid and protein can convert to carbohydrate as fuel for energy, foraging to protein sources can meet the nutrient requirement for growth and can be used as an energy source when carbohydrate is not sufficient (Chapman 1998).

During both early and peak colony growth, *P. chinensis* collected all food types (carbohydrate, lipid, and protein). At the peak of the worker population and occurrence of reproductive alates, *P. chinensis* showed significant preference for protein. This information should be useful when designing bait management strategies for *P. chinensis*.

CHAPTER THREE

EFFICACY OF SELECTED BAIT PRODUCTS AGAINST AN INVASIVE ANT, *PACHYCONDYLA CHINENSIS* (EMERY) (HYMENOPTERA: FORMICIDAE)

Introduction

Pachycondyla chinensis (Emery) is commonly known as the Asian needle ant. After it was first found in Decatur, Georgia, USA by Vanderford, it also was found in a number of locations including Wilmington, Newbern, Washington, and Elizabeth City, North Carolina, Norfolk, and Petersburg, Virginia, and Richmond, Georgia (Smith 1934). In 2007, Paysen collected specimens of *P. chinensis* from multiple counties in South Carolina and identified this species in North Carolina, Tennessee and Alabama, USA through confirmed reports and by survey. He predicted the extending range of *P. chinensis* in the southeastern USA. By 2010, *Pachycondyla chinensis* was described in most eastern states from Connecticut to the northernmost part of Florida (Guénard and Dunn, 2010).

The threat to ecological systems and diversity was predicted and shown by Paysen (2007) and further studied by Guénard and Dunn (2010). In both studies the presence of *P. chinensis* was negatively correlated with composition, abundance and diversity of native ant species. Rodriguez-Cabal et al. (2011) also reported *P. chinensis* was associated with the disruption of ant-plant seed dispersal mutualism, and potentially responsible for reduced abundance of ant-dispersed plants (Rodriguez-Cabal et al. 2011). *Pachycondyla chinensis* is native to temperate and subtropical regions of Eastern Asia countries such as China, Korea and Japan (Creighton 1950, Brown 1958, Lee et al. 2009). The sting of *P. chinensis* was known to cause local skin reactions and serious systematic reactions in Korea and Japan (Kwon et al.1999, Fukuzawa 2002, Leath 2006). Characterization of major allergens was studied in anaphylaxis patients stung by *P. chinensis* in Korea (Yun et al. 1999, Kim et al. 2001, Cho et al. 2002, Lee et al. 2009). When Vanderford first documented *P. chinensis* in the USA, he also had reports of occasional stings by this ant (Smith 1934). Complaints of painful and itchy stings by *P. chinensis* were first reported from zookeepers of the Greenville Zoo (Greenville, Greenville Co.) in South Carolina in 2004 (Nelder et al. 2006). Increasing sting cases were reported recently in South Carolina. Victims were commonly stung while working in the garden or on landscapes, or engaged in other outdoor activities (Nelder et al. 2006, Jackson 2012).

The nests of *P. chinensis* were found in rotten logs, under objects where moisture is stable, and in the soil beneath stones (Paysen 2007, Smith 1934). In the urban environment, it was commonly found by sidewalks, in cracks and crevices around buildings, and flower beds, around tree bases where moisture is held by tree holes and mulch, and under ornamental landscape objects and flower pots (Paysen 2007, Guénard and Dunn 2010, personal observation). Frequent human activity occurs in these areas.

Control recommendations for *P. chinensis* are only speculative. General chemical control against pest ants can be categorized as colony treatment, perimeter treatment, and toxic bait application. Individual mound treatment and spot treatment targeting nest sites and areas of high activity are often used. Precise locations of nests and active trails are important aspects for using these techniques successfully. *Pachycondyla chinensis* nest without building a mound, and their nest entrances are cryptic and multiple. Nests of *P.*

chinensis are hidden and nest materials often are cluttered wood piles, debris and construction objects in the urban environment. Foraging scouts search for food sources individually. Control methods treating individual mounds and ant trials are impractical against *P. chinensis*.

Pachycondyla chinensis nests are either polydomous or monodomous depending on the habitat available (Paysen 2007). The majority of nests were polygynous in Paysen's study in the Clemson area (South Carolina, USA), with as much as 18% of the colony being queens, showing remarkable reproductive potential (Paysen 2007). Polydomous nests are somehow scattered. *Pachycondyla chinensis* changes nest location rapidly after a disturbance (personal observation). These nesting characteristics of *P. chinensis* increase the difficulty of identifying the precise location of all colonies. Without precisely targeting the ants, chemical control using contact insecticides formulated as a dust, liquid or spray can be inefficient and costly.

Perimeter treatment providing a barrier around the structure or food source with a residual insecticide can be used to prevent pest ants from invasion. Preferred nest sites of *P. chinensis*, such as rotten logs, leaf litter, and garden ornaments, are located outdoors in urban environment. Ants are not inclined to invade buildings, but may be brought in accidently by humans or may fly into structures in the swarmer stage. Exclusion of *P. chinensis* using a perimeter treatment is not applicable.

Baiting is an important control method for pest ants with many advantages. It is easy to use, is less likely to be impacted by soil type or precise nest location than insecticidal spray, provides sufficient long-term control with a proper combination of treatments, is distributed to all members of the colony, specifically targets attracted species, and requires only a small amount of toxicant (Lucas and Invest 1993, Silverman and Rouslton 2001, Stanley 2004).

Klotz et al. (1997) stated that bait consists of four components: an attractant, a palatable carrier, a toxicant, and other materials added for reasons of formulation. The attractants in ant baits are food or a pheromone which encourages ants to bring the bait back to the nest. In the food preference study, *P. chinensis* visited protein significantly more than carbohydrate and lipid at peak population in late summer (Chapter 2). However, they also visited carbohydrate and lipid, and the percentage of ants visiting protein was not significantly greater than carbohydrate or lipid during early stages of population growth.

The matrix made up of carrier and additive has an effect on bait acceptance and delivery (Silverman and Roulston 2001). There are many commercial ant baits on the market described as oil bait, protein bait, bait for sweet ants, or bait containing a blend of foods. The specific attractant in commercial baits often is not revealed. Besides different types of attractants, these baits are formulated into a variety of matrices and particle sizes. There are no commercial baits specifically labeled for *P. chinensis* at this time.

The relative effectiveness of available commercial baits on the market against *P. chinensis* is unknown. The objective of this study is to evaluate the relative effectiveness of selected bait products for control of *P. chinensis*. By evaluating selected commercial bait products and combining knowledge of *P. chinensis* biology, more practical recommendations for management of *P. chinensis* can be made.

Based on the results of the food preference study, the hypothesis is that products designed to attract both sweet and protein feeding ants will be more effective for the control of *P. chinensis*.

Materials and Methods

Bait Product Survey

A survey of ant bait products was conducted to determine available active ingredients, formulations, and manufacturers. The type of formulation and active ingredient were taken into consideration.

Pachycondyla chinensis was described opportunistically feeding on live or dead insects, and decaying fruit. They prey on small live arthropods and show a preference for *Reticulitermes virginicus* (Banks) among potential prey around nests (Bednar and Silverman 2011). Another well-studied, invasive ant, *Linepithema humile* (Mays), uses honeydew produced by Hemipterans as an important food source. *Linepithema humile* was demonstrated to handle liquid bait more efficiently than gel bait (Silverman and Roulston, 2001). *Pachycondyla chinensis* is not considered a honeydew feeder. In this study, non-liquid bait products primarily were chosen to evaluate efficacy against *P. chinensis.* Seven products were selected including five active ingredients, and three types of formulations (Table 3.1).

Table 3.1 Bait products used in study to evaluate efficacy against *Pachycondyla chinensis* (Emery)

Product	Active Ingredient	Bait Formulation	Manufacturer
Advion® fire ant bait[1]	Indoxacarb (0.045%)	Granules	DuPont™
Advion® granule[2]	Indoxacarb (0.05%)	Granules	DuPont™
Advion® gel[3]	Indoxacarb (0.22%)	Gel	DuPont™
Advance®[4]	Abamectin (0.011%)	Granules	BASF Corporation
Optigard®[5]	Thiamethoxam (0.01%)	Gel	Syngenta Crop Protection
Maxforce® complete[6]	Hydramethylnon (1.00%)	Granules	Bayer Environmental
Maxforce® quantum[7]	Imidacloprid (0.03%)	Liquid	Bayer Environmental

Abbreviations of product full name: [1]Advion® fire ant bait (DuPont™, Nemours and Company, Wilmington, Delaware), [2]Advion® Insect Granule (DuPont™, Nemours and Company, Wilmington, Delaware), [3]Advion® ant gel (DuPont™, Nemours and Company, Wilmington, Delaware), [4]Advance® 375A Select Granular Ant Bait (BASF Corporation, St. Louis, Missouri), [5]Optigard® Ant gel Bait (SYNGENTA CROP Protection, Greensboro, North Carolina), [6]Maxforce® Complete Brand Granular Insect Bait (Bayer Environmental, Research Triangle Park, North Carolina), [7]Maxforce® Quantum Ant Bait(Bayer Environmental, Research Triangle Park, North Carolina). Product names mentioned in the following article were used as the abbreviated format.

1. Choice/No Choice Bait Study - Laboratory

Colony Collection

Nests of *P. chinensis* were collected in the Clemson University Experimental Forest (South Carolina, USA) by removing soil and material surrounding the nest with a shovel. Nests were considered different colonies when they were established farther than 20 m apart. Individual nests were placed separately in 18.9 liter plastic buckets and returned to the laboratory (Clemson University Urban Entomology Laboratory, South Carolina, USA).

Colonies were maintained in plastic containers (58 cm x 38 cm x 15 cm). An aluminum- foil-wrapped test tube (15 mm in diameter, 150 mm in length) plugged with a moist sponge (approximately 1 cm x 1cm x 10cm) was placed in the container. The sponge provided higher than ambient humidity inside the test tube, and acted as a water source for ants. Water was added to the sponge *ad libitum*. Debris containing the collected ants was spread in the container to dry out and larger pieces of wood debris were removed after ants vacated it during drying. Ants moved into the test tube seeking moisture, and used it as a nest. Most larvae were attached to the inner wall of the test tube. Ants remaining nested under collected pieces of wood were maintained without disturbance. Colonies were maintained at 22 ± 3ºC, RH 65% and fed a constant diet of termites.

Experimental Design

Five larvae and 20 workers were collected from the laboratory-maintained colonies and held in a glass Petri dish (5 cm in diameter). Larvae were picked up using soft-tipped forceps, and workers were collected by aspirator. A water-soaked sponge was inserted into a small plastic sleeve (2 cm in length, 1.9 cm in diameter) and placed in the center of the Petri dish as nesting area. Termite workers were used as food. The bait was provided in a half-cut weigh boat (side length of top 2.5 cm, bottom 1.5 cm). Eight treatments including seven products and one control were conducted to determine efficacy in both choice and no-choice trials (Table 3.1). Ants were allowed to habituate in the glass Petri dish for 48 hours before bait was provided. Each Petri dish setup was an experimental unit. Eight Petri dishes which were randomly assigned to the eight treatments were one replicate. Three termite workers were added in each dish at the beginning of the habituation period to equalize food access over all experimental units.

Ants in each replicate were from the same colony. A choice test was performed by providing food and bait in the treatment dish. Ants had a choice between preferred termites or bait. Control ants were offered termites only. A total number of five replicates were performed using five colonies. In the no-choice test, ants were offered only bait in the treatment and only termites in the control. A total number of six replicates were performed using four colonies. Food and water were refreshed every day for 14 days. Bait products were refreshed every three days. Ant mortality was recorded daily for 14 days.

Statistical Analysis

Tests were conducted as a complete block design. Colony was considered as a block effect. The number of replicates conducted in each block varied depending on the colony size. Analysis of variance was performed on arcsine square root transformed data in the study comparing the daily mortality of each treatment (PROC GLM, SAS institution 9.3 2011). Fisher's Least Significant Difference test was used to compare the difference in mean daily mortality between each two treatments.

2. Choice/No Choice Bait Study - Field Study

Study Site and Material

Four locations were chosen in the area around Clemson (South Carolina, USA). They were the campus of Clemson University (Pickens County, South Carolina, 34º 40’ 44” N, 82º 49’ 55” W), Woodburn Historical Plantation House (Anderson County, South

Carolina, 34º 38’ 26” N, 82º 47’ 44” W), and two residence houses (Anderson County, South Carolina, 34º 38’ 25.29” N, 82º 49’ 03.97” W; Pickens County,, South Carolina, 34º 41’ 04.44” N, 82º 47’ 24.85” W). An area with actively foraging *P. chinensis*, and potential nesting materials was defined as a site. Each site was an experimental unit, and located at least 10 meters apart without overlapping foraging resources. Bait products used were the same as in the laboratory study (Table 3.1). The field test was conducted from June to Aug 2012 for ten weeks.

Four locations were separated into three different types of environments: 1) Campus, campus of Clemson University was full of activity during the daytime and maintained regularly with irrigation, mulching and removal of fallen leaves, 2) Woodburn, Woodburn Historical Plantation House was a well-maintained older structure surrounded by heavy vegetation. Occasional human activity occurred during tours and catered events on the grounds, 3) House, two residence houses were also surrounded by heavy vegetation. Yard maintenance, irrigation, mulching, and removal of debris were unpredictable.

Eight sites which received treatments, including seven bait product and one control, were considered as a replicate. Two replicates were performed on the campus of Clemson University. Woodburn Historical Plantation was one replicate, and the two residence houses were considered as one replicate. A total of four replicates were conducted. Both residence houses included control sites and an extra control site was established on campus because sites were widely separated. A total of 34 sites were identified for use.

Monitoring

There are several methods reported to evaluate ground-dwelling or leaf-litter ant populations including hand sampling, bait trapping, Winkler sacks, and pitfall trapping (Majer 1978, Fisher et al. 2000, Gotelli et al. 2011, Nunes et al. 2011, Rice 2012). Pitfall trapping was used in this study. It is a useful measurement to estimate biomass within an effective foraging range and travel distance (Gotelli et al. 2011, Nunes 2011). Pitfall traps of the right design, size, placement, and duration in the field provided undisturbed monitoring for evaluating population changes in *P. chinensis* after bait application.

Pitfall traps consisted of a test tube (15 cm long, 18 mm in diameter) half-filled with unscented antifreeze containing ethyl alcohol, glycol, denaturant, colorant and water (Arctic Ban, Camco Manufacturing Inc, Greensboro, NC), inserted into a PVC sleeve (Majer 1978, Fisher et al. 2000, Nunes 2011). Test tube and PVC sleeve were put into the soil together. The top was flush with the soil line and covered with a square (10 cm^2) of vinyl flooring to prevent flooding from rainfall and irrigation. The cover was held in place by a nail (8.9 cm in length) and a space of a few centimeters separated it from the ground to allow easy access for ants.

Experiment Design

Three pitfall traps were installed at each site. Each pitfall trap placement was approximately one meter apart in a *P. chinensis* active foraging area. Each week the entire contents of the trap was collected into a puncture-proof sample bag and returned to the laboratory for counting. The test tube was refilled with fresh antifreeze after contents were collected.

Pitfall trapping data were collected a week before baits were applied in late May, 2012. To assign treatments to sites, the number of *P. chinensis* counted from pitfall traps was sorted from high to low, and grouped to allow for random assignment of treatments within each group size (Table 3.2); each treatment was assigned to a replicate in such a way as to balance the population numbers across treatments.

Table 3.2 Weekly number of *Pachycondyla chinensis* (Emery) workers captured in pitfall traps at each study site before treatments were applied (May, 2013)

Environmental types	Replicate	T1	T2	T3	T4	T5	T6	T7	T8	
Campus	1	61	209	85	119	284	64	57	440	14[1]
	2	82	43	26	48	34	35	87	20	
Woodburn	3	108	18	59	60	83	78	87	39	
House	4	17	27	146	49	41	280	52	25	16[1]

[1]Extra control sites were set up at the campus location and P&E house locations increasing the replication of the control to five.

Bait products were applied according to label directions at the highest rate (Table 3.3). Advion® fire ant bait was applied evenly to the perimeter of the nest at a rate of 14.175 g per nest. The amount of chemical used was at the rate for an individual colony and was distributed in a one-foot wide band around nests. Advion® granule was broadcasted at a rate of 208.656 g/9 m^2 plot (3 m by 3 m), with the nest in the center of the plot. Advion® gel was provided in two stations for each site. The station was a clear plastic, refillable container (Maxforce®, Bayer Environmental, Research Triangle Park, North Carolina). They were relabeled with chemical information and contact information. Advance® was applied at a 56.699 g rate to the nest perimeter. It was used as an individual colony treatment. Optigard® was applied in the same way as T3. The stations were also relabeled with chemical and contact information. Maxforce® complete was placed into a refillable station. One station was placed on the ground close to the nest at each site. Maxforce® quantum was made available in refillable stations with two stations placed around each nest site. No application was applied at control sites.

Bait products were reapplied according to label directions (Table 3.3). Baits applied in stations were inspected and refreshed every week when collecting pitfall trap samples. Reapplication of the broadcasted bait was initiated after collecting pitfall traps. Advion® granule and Advance® were reapplied after weeks 2, 4 and 7. Advion® fire ant bait was reapplied after weeks 2 and 7.

Table 3.3 Application method of each bait product used in a field study to investigate relative efficacy against *Pachycondyla chinensis* (Emery)

	Application Method	
	Broadcast application reapplied according to label	Applied in station-refreshed weekly
Products	T1(14.175g)	T3
	T2 (208.656g)	T5
	T4 (56.699g)	T6
		T7

T1: Advion® fire ant bait; T2: Advion® granule; T3: Advion® gel; T4: Advance®; T5: Optigard®; T6: Maxforce® complete; T7: Maxforce® quantum.

Data analysis

The type of environment of the experimental locations was considered as block effects. The blocks included Campus, Woodburn and House (Table 3.2). The number of *P. chinensis* collected in pitfall traps at each site every week was compared with the weekly number before treatments were applied. The percentage of *P. chinensis* population change at each site was evaluated by analysis of variance and followed by a Tukey-Kramer test to separate means if appropriate (PROC MIXED SAS Institution 9.3 2011).

<u>Result</u>

1. Choice/No Choice Study-Laboratory

In both choice and no-choice laboratory tests, the mortality of *P. chinensis* exposed to selected bait products in 14 days was transformed into arsine square root values for analysis of variance to better fit a normal curve. The results showed a significant difference in mortality in 14 days between treatments in both choice and no-choice tests (Table 3.4).

Table 3.4 Analysis of variance using arcsine square root transformed data of mortality of *Pachycondyla chinensis* (Emery) exposed for 2 weeks to selected commercial bait products in laboratory tests.

Day	Choice			No-choice		
	DF	F value	P value	DF	F value	P value
1	7, 28	17.63	<0.0001	7, 37	10.56	<0.0001
2		34.54	<0.0001		18.98	<0.0001
3		57.87	<0.0001		26.50	<0.0001
4		50.63	<0.0001		24.69	<0.0001
5		38.19	<0.0001		34.62	<0.0001
6		45.11	<0.0001		53.95	<0.0001
7		45.82	<0.0001		49.80	<0.0001
8		42.46	<0.0001		48.97	<0.0001
9		36.38	<0.0001		56.78	<0.0001
10		36.00	<0.0001		61.26	<0.0001
11		29.35	<0.0001		60.68	<0.0001
12		30.20	<0.0001		54.28	<0.0001
13		27.87	<0.0001		48.77	<0.0001
14		27.25	<0.0001		55.58	<0.0001

After 14 days, the survival rate in the control was close to 95% in the choice test and 90% in the no-choice test (Tables 3.5; 3.6; 3.7; 3.8). In the choice test, the mortality of *P. chinensis* exposed to Advion® fire ant bait, Advance® and Maxforce® complete was

100% and not significantly different from ants exposed to Advion® gel which was 90.16%. Optigard® yielded 43.30% mortality and was not significantly different from Maxforce® quantum (39.78%). Advion® granule was not significantly different from the control (Tables 3.5; 3.6). In the no-choice test, those bait products that achieved 100% mortality in the choice test were equally successful, and Advion® gel (96.62%) also was not significantly different. However, Optigard® yielded 86.69% mortality which was not significantly different from Advion® gel, but was significantly different from Maxforce® quantum. Every treatment in the no-choice test yielded significantly higher mortality than the control (Tables 3.7; 3.8).

In the choice test, Advion® fire ant bait reached 100% mortality on the third day and was significantly higher than the other treatments (Fig. 3.1). Advance® and Maxforce® complete reached mortality that was not significantly different from Advion® fire ant bait from day 4. The mortality of ants exposed to Advion® gel was not significant from Advion® fire ant bait from day 12.

In the no-choice test, ants were provided with bait only in the treatment arena. Maxforce® complete yielded 100% mortality on day 5, but was not significantly different from Advion® fire ant bait and Advance® during the 14-day test (Fig. 3.2). The mortality of ants exposed to Advion® gel was not significantly different from those products reaching 100% mortality from day 8 and not significantly different from Optigard® during the 14-day test.

Table 3.5 Mean (±SE) percentage mortality of *Pachycondyla chinensis* (Emery) exposed to selected commercial bait products in a choice test for 14 days (Part 1).

Day	Treatment			
	T1	T2	T3	T4
1	39.47 ± 10.02a	0.00 ± 0.00c	3.95 ± 6.51c	16.98 ± 14.54b
2	80.68 ± 19.73a	0.00 ± 0.00d	14.13 ± 8.89c	56.48 ± 16.45b
3	100.00 ± 0.00a	1.11 ± 2.49e	32.92 ± 18.24c	90.74 ± 8.30b
4	100.00 ± 0.00a	8.89 ± 19.88de	44.05 ± 21.71b	94.79 ± 6.45a
5	100.00 ± 0.00a	14.44 ± 29.29de	49.90 ± 23.34b	96.89 ± 2.84a
6	100.00 ± 0.00a	14.44 ± 29.29de	56.90 ± 13.83b	97.89 ± 2.88a
7	100.00 ± 0.00a	16.56 ± 31.23de	63.90 ± 15.10b	98.95 ± 2.35a
8	100.00 ± 0.00a	16.56 ± 31.23de	71.02 ± 20.53b	98.95 ± 2.35a
9	100.00 ± 0.00a	17.67 ± 33.71de	76.13 ± 21.56b	98.95 ± 2.35a
10	100.00 ± 0.00a	17.67 ± 33.71de	85.30 ± 21.43b	98.95 ± 2.35a
11	100.00 ± 0.00a	19.89 ± 38.66de	85.30 ± 21.43b	98.95 ± 2.35ab
12	100.00 ± 0.00a	19.89 ± 38.66cd	89.16 ± 15.91a	100.00 ± 0.00a
13	100.00 ± 0.00a	22.11 ± 37.95cd	89.16 ± 15.91a	100.00 ± 0.00a
14	100.00 ± 0.00a	22.11 ± 37.95cd	90.16 ± 16.51a	100.00 ± 0.00a

Number followed by the same latter within each row is not significantly different
T1: Advion® fire ant bait; T2: Advion® granule; T3: Advion® gel; T4: Advance®.

Table 3.6 Mean (± SE) percentage mortality of *Pachycondyla chinensis* (Emery) exposed to selected commercial bait products in a choice test for 14 days (Part 2).

Day	Treatment			
	T5	T6	T7	T8
1	1.95 ± 2.67c	13.98 ± 11.57b	1.00 ± 2.24c	0.00 ± 0.00c
2	3.95 ± 4.17cd	79.83 ± 26.23a	4.00 ± 4.18cd	0.00 ± 0.00d
3	8.95 ± 8.25de	85.00 ± 28.06b	11.33 ± 4.62d	0.95 ± 2.13e
4	19.06 ± 13.80c	90.00 ± 22.36a	14.44 ± 7.08cd	0.95 ± 2.13e
5	30.06 ± 26.85bc	93.00 ± 15.65a	17.56 ± 10.96cd	1.95 ± 2.67e
6	34.24 ± 23.62bc	96.00 ± 8.94a	22.78 ± 11.10cd	2.95 ± 2.70e
7	38.35 ± 26.60c	98.00 ± 4.47a	26.11 ± 17.70cd	2.95 ± 2.70e
8	38.35 ± 26.60c	99.00 ± 2.24a	28.33 ± 22.39cd	3.95 ± 4.17e
9	39.35 ± 28.58c	100.00 ± 0.00a	30.33 ± 23.02cd	3.95 ± 4.17e
10	40.30 ± 27.39c	100.00 ± 0.00a	32.33 ± 22.87cd	3.95 ± 4.17e
11	40.30 ± 27.39c	100.00 ± 0.00a	34.56 ± 27.18cd	4.90 ± 4.88e
12	40.30 ± 27.39b	100.00 ± 0.00a	35.67 ± 29.41bc	4.90 ± 4.88d
13	42.30 ± 29.48b	100.00 ± 0.00a	37.78 ± 30.88bc	4.90 ± 4.88d
14	43.30 ± 29.68b	100.00 ± 0.00a	39.78 ± 29.33bc	4.90 ± 4.88d

Number followed by the same latter within each row is not significantly different
T5: Optigard®; T6: Maxforce® complete; T7: Maxforce® quantum; T8: Control.

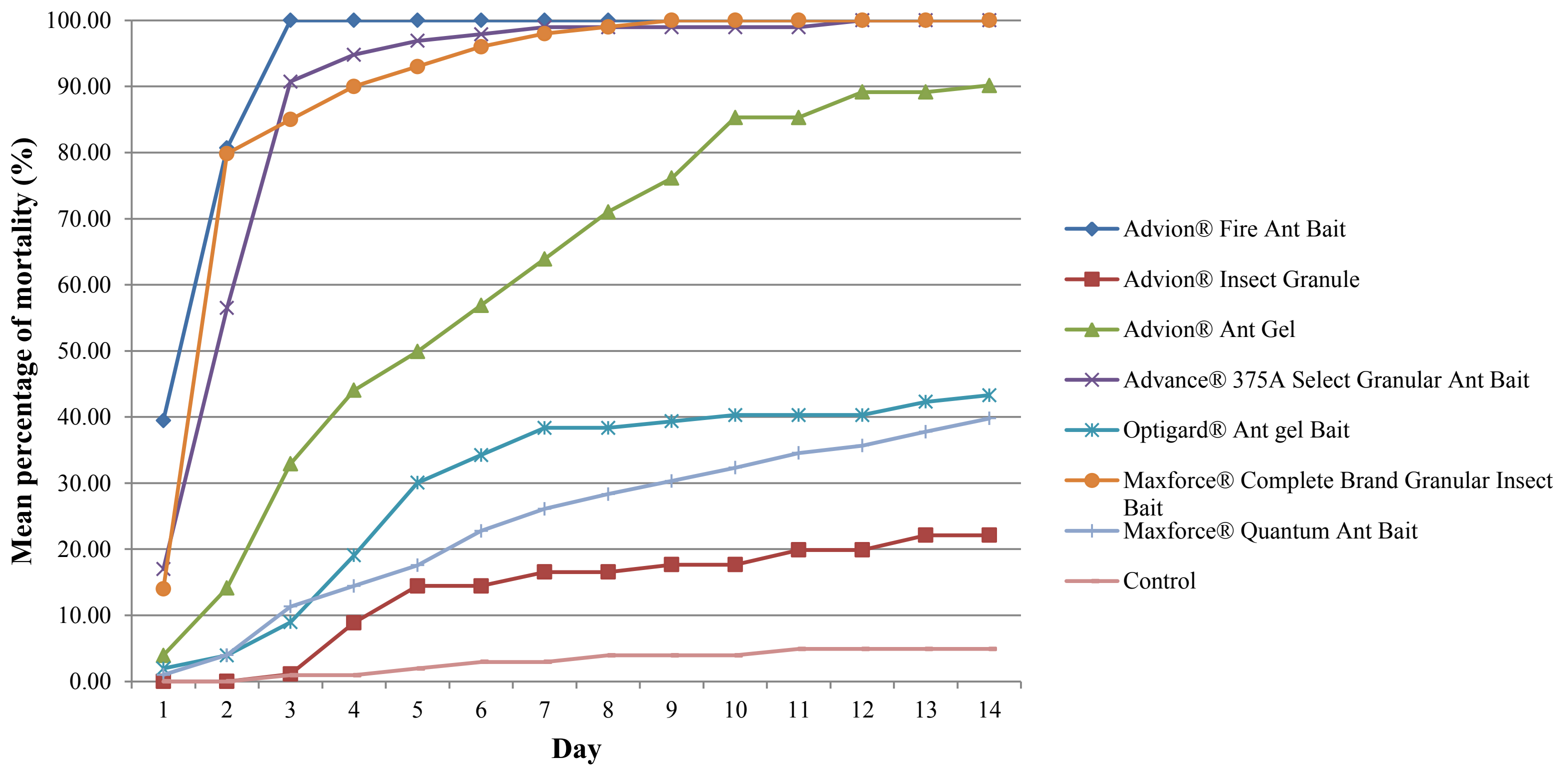

Figure 3.1 Mean percentage mortality of *Pachycondyla chinensis* (Emery) exposed to selected commercial bait products in a choice test for 14 days. Five colonies were used for each treatment.

Table 3.7 Mean (± SE) percentage mortality of *Pachycondyla chinensis* (Emery) exposed for 2 weeks to selected commercial bait products in a no-choice test (Part 1).

Day	Treatment			
	T1	T2	T3	T4
1	45.64 ± 35.98a	0.00 ± 0.00d	13.90 ± 15.09bc	21.05 ± 8.81b
2	81.57 ± 40.05a	0.98 ± 2.40c	30.29 ± 23.82b	71.93 ± 22.49a
3	83.33 ± 40.82a	0.98 ± 2.40c	48.62 ± 25.46b	94.74 ± 8.81a
4	83.33 ± 40.82a	1.86 ± 2.88c	60.29 ± 29.50b	96.49 ± 6.37a
5	85.00 ± 36.74a	5.32 ± 3.34c	71.17 ± 27.10b	97.37 ± 4.40a
6	93.33 ± 16.33a	7.08 ± 5.42c	79.68 ± 19.35bb	97.37 ± 4.40a
7	98.33 ± 4.08a	10.59 ± 9.38c	84.77 ± 14.71b	98.25 ± 4.30a
8	98.33 ± 4.08a	13.38 ± 8.83de	91.57 ± 7.18bc	98.25 ± 4.30ab
9	100.00 ± 0.00a	15.05 ± 9.01cd	93.24 ± 7.87ab	98.25 ± 4.30a
10	100.00 ± 0.00a	16.76 ± 9.41cd	93.24 ± 7.87ab	98.25 ± 4.30a
11	100.00 ± 0.00a	21.16 ± 12.51cd	95.74 ± 6.13ab	100.00 ± 0.00a
12	100.00 ± 0.00a	27.30 ± 15.75c	96.62 ± 4.24ab	100.00 ± 0.00a
13	100.00 ± 0.00a	31.01 ± 16.11c	96.62 ± 4.24ab	100.00 ± 0.00a
14	100.00 ± 0.00a	34.44 ± 16.17c	96.62 ± 4.24ab	100.00 ± 0.00a

Number followed by the same latter within each row is not significantly different
T1: Advion® fire ant bait; T2: Advion® granule; T3: Advion® gel; T4: Advance®.

Table 3.8 Mean (± SE) percentage mortality of *Pachycondyla chinensis* (Emery) exposed for 2 weeks to selected commercial bait products in a no-choice test (Part 2).

Day	Treatment			
	T5	T6	T7	T8
1	3.26 ± 6.03cd	25.83 ± 10.68ab	2.64 ± 2.89cd	0.93 ± 2.27d
2	12.13 ± 13.39bc	81.67 ± 13.29a	2.64 ± 2.89c	1.85 ± 4.54c
3	42.74 ± 32.94b	95.00 ± 6.32a	6.06 ± 6.01c	2.69 ± 4.59c
4	56.11 ± 35.37b	96.67 ± 4.08a	6.06 ± 6.01c	2.69 ± 4.59c
5	66.96 ± 34.21b	100.00 ± 0.00a	9.58 ± 6.69c	3.61 ± 6.70c
6	70.29 ± 33.34b	100.00 ± 0.00a	9.58 ± 6.69c	3.61 ± 6.70c
7	74.55 ± 34.58b	100.00 ± 0.00a	12.91 ± 12.03c	3.61 ± 6.70c
8	79.47 ± 31.07c	100.00 ± 0.00a	22.17 ± 21.78d	4.44 ± 6.47e
9	82.80 ± 23.58b	100.00 ± 0.00a	27.18 ± 25.99c	4.44 ± 6.47d
10	86.14 ± 17.90b	100.00 ± 0.00a	32.20 ± 27.78c	6.94 ± 5.72d
11	86.14 ± 17.90b	100.00 ± 0.00a	36.52 ± 26.47c	8.61 ± 6.45d
12	86.89 ± 17.78b	100.00 ± 0.00a	42.57 ± 28.47c	10.28 ±7.33d
13	86.89 ± 17.78b	100.00 ± 0.00a	46.92 ± 30.22c	10.28 ± 7.33d
14	86.89 ± 17.78b	100.00 ± 0.00a	50.41 ± 28.03c	10.28 ± 7.33d

Number followed by the same latter within each row is not significantly different
T5: Optigard®; T6: Maxforce® complete; T7: Maxforce® quantum; T8: Control.

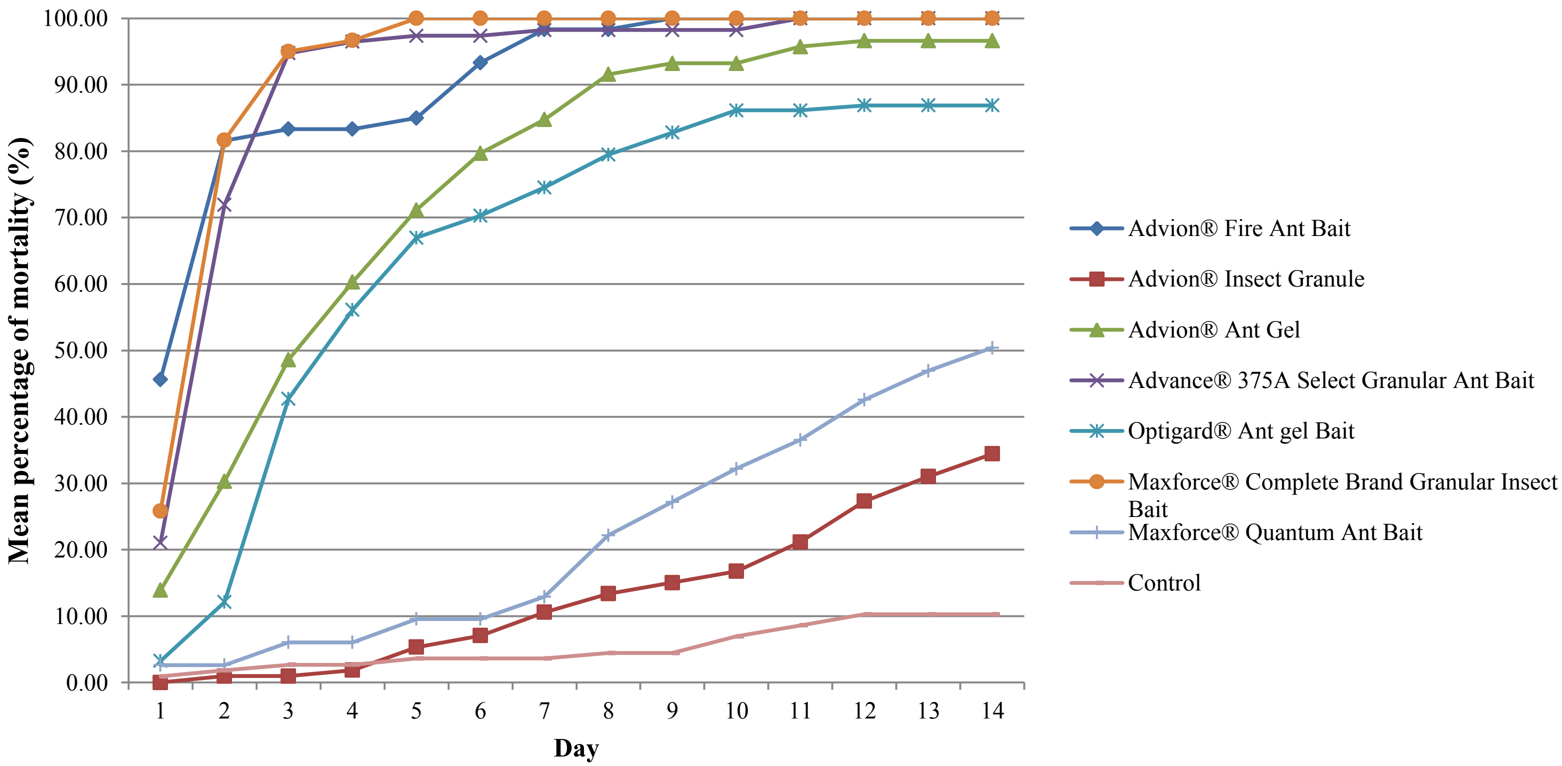

Figure 3.2 Mean percentage mortality of *Pachycondyla chinensis* (Emery) exposed to selected commercial bait products in a no-choice test for 14 days. Six colonies were used for each treatment.

2. Field Study

The ANOVA results showed significant differences in the mean percentage of *P. chinensis* population change at each site treated with the seven selected bait products in week 1 only ($F=3.42$; $df=3, 24$; $P=0.011$) (Fig. 3.3). There were no significant differences in the mean percentage of *P. chinensis* population change between treatments in the other nine weeks ($P>0.05$)

In the first week after treatment, sites treated with Advion® fire ant bait and Advion® granule showed significantly higher population reduction than Maxforce® complete, which showed there was an increasing mean percentage of population change (22.78%). There were no significant differences between the other treatments ($P>0.05$).

Overall, in the first three weeks, the mean percentage of *P. chinensis* population change was reduced at control sites and every treatment site except those treated with Maxforce® complete (Fig. 3.3; 3.4). The population changes in the remaining seven weeks were varied (Figs. 3.3; 3.4; 3.5; 3.6; 3.7). The population increased dramatically at control sites during weeks 4, 5 and 6 which ranged from late June to mid-July, and it also increased at sites treated with Advion® granule, Optigard®, Maxforce® complete, and Maxforce® quantum.

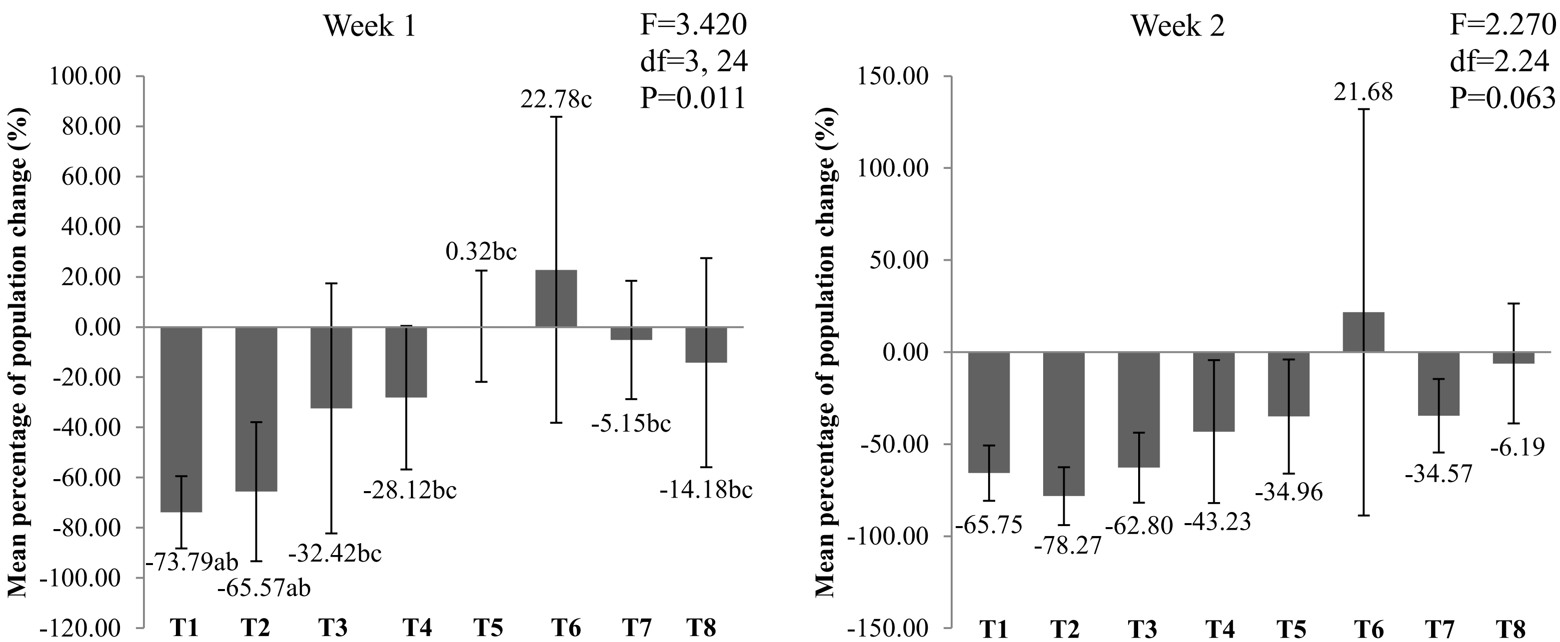

Figure 3.3 (Mean ± SE) percentage *Pachycondyla chinensis* (Emery) population reduction in weeks 1and 2 when given access to selected bait products in the field; four replicates were conducted in three locations for each treatment. The series columns from left to right are: T1-Advion® fire ant bait; T2-Advion® granule; T3-Advion® gel; T4-Advance®; T5-Optigard®; T6-Maxforce® complete; T7- Maxforce® quantum; and T8-Control.

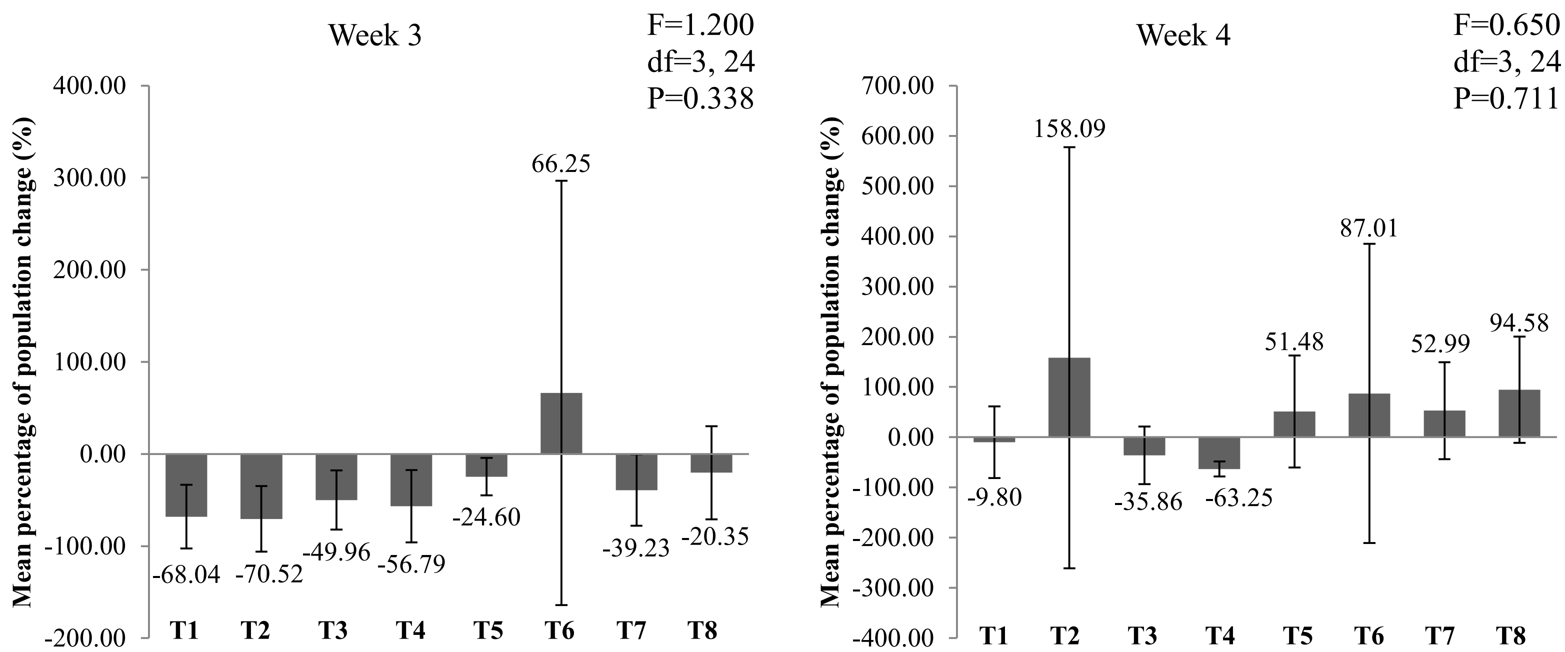

Figure 3.4 Mean (± SE) percentage *Pachycondyla chinensis* (Emery) population reduction in weeks 3 and 4 when given access to selected bait products in the field; four replicates were conducted in three locations for each treatment. The series columns from left to right are: T1-Advion® fire ant bait; T2-Advion® granule; T3-Advion® gel; T4-Advance®; T5-Optigard®; T6-Maxforce® complete; T7- Maxforce® quantum; and T8-Control.

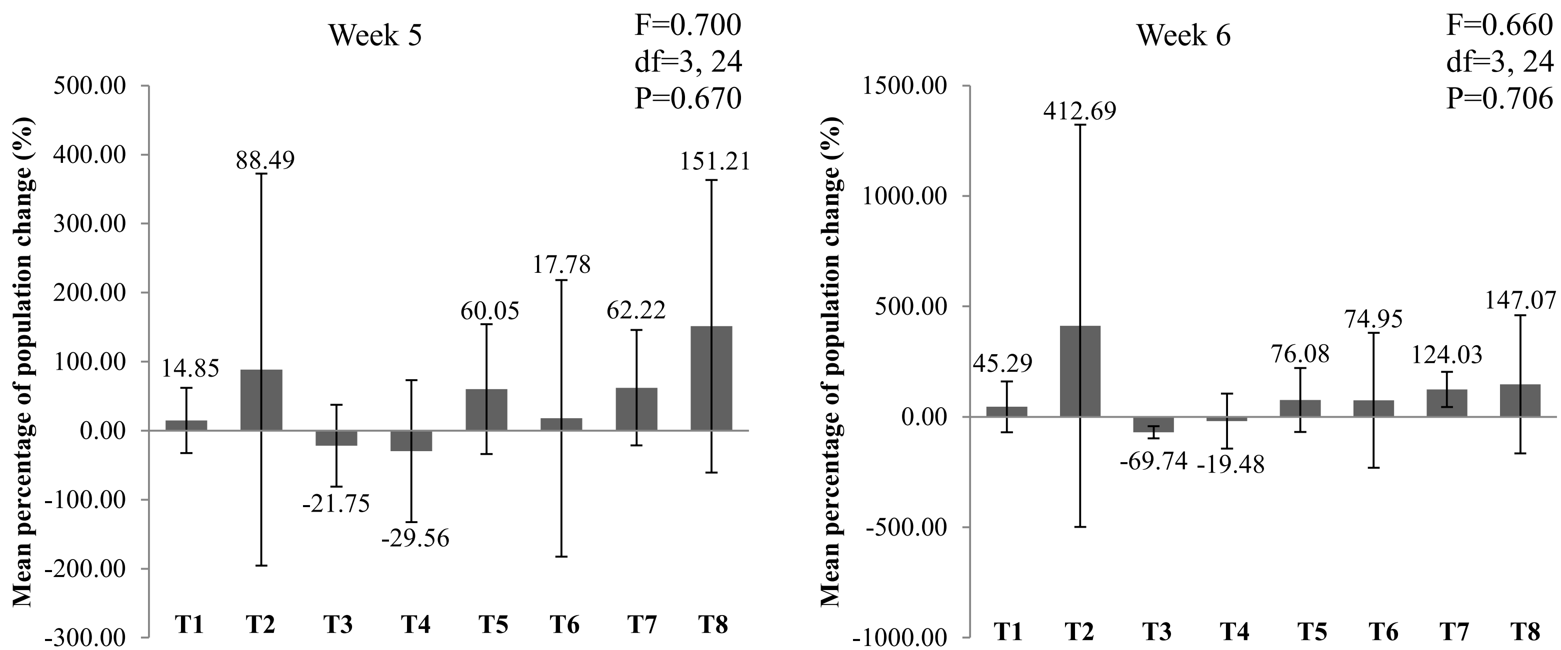

Figure 3.5 Mean (± SE) percentage *Pachycondyla chinensis* (Emery) population reduction in weeks 5 and 6 when given access to selected bait products in the field; four replicates were conducted in three locations for each treatment. The series columns from left to right are: T1-Advion® fire ant bait; T2-Advion® granule; T3-Advion® gel; T4-Advance®; T5-Optigard®; T6-Maxforce® complete; T7- Maxforce® quantum; and T8-Control.

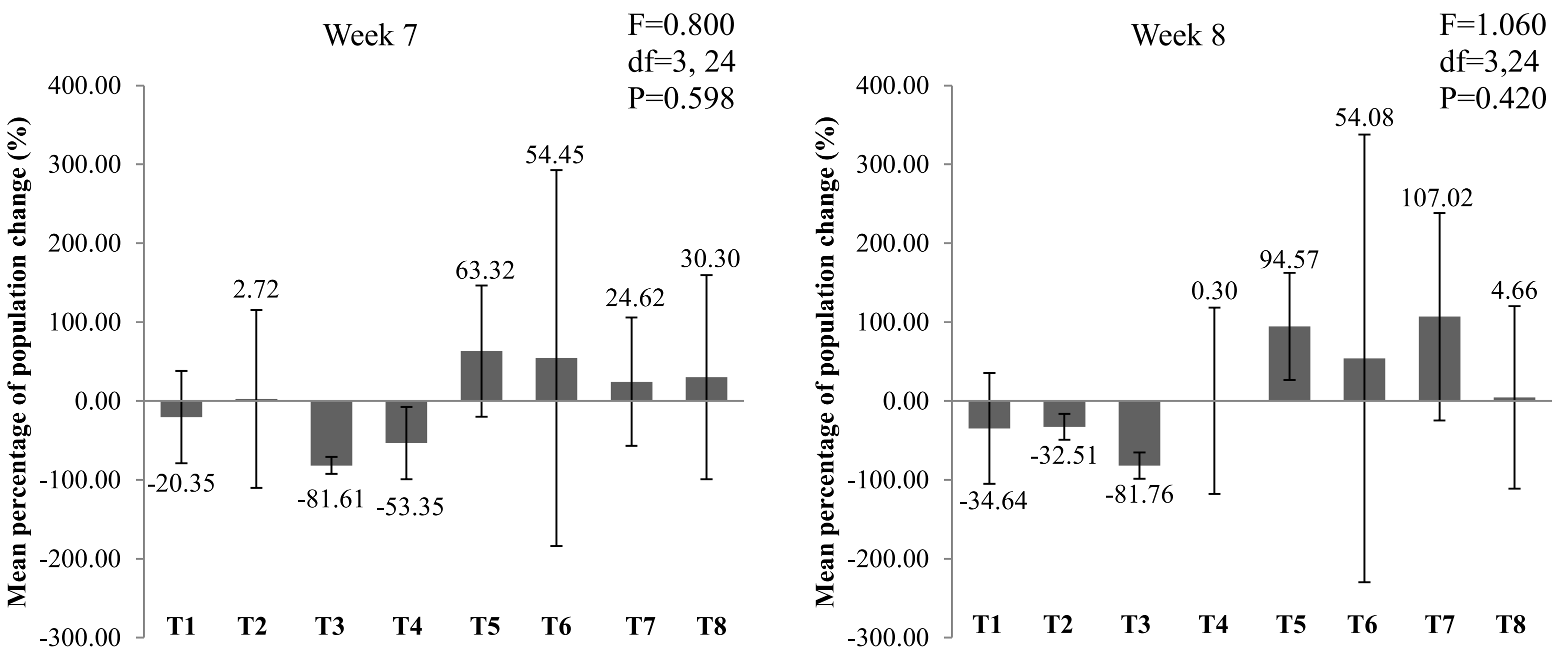

Figure 3.6 Mean (± SE) percentage *Pachycondyla chinensis* (Emery) population reduction in weeks 7 and 8 when given access to selected bait products in the field; four replicates were conducted in three locations for each treatment. The series columns from left to right are: T1-Advion® fire ant bait; T2-Advion® granule; T3-Advion® gel; T4-Advance®; T5-Optigard®; T6-Maxforce® complete; T7- Maxforce® quantum; and T8-Control.

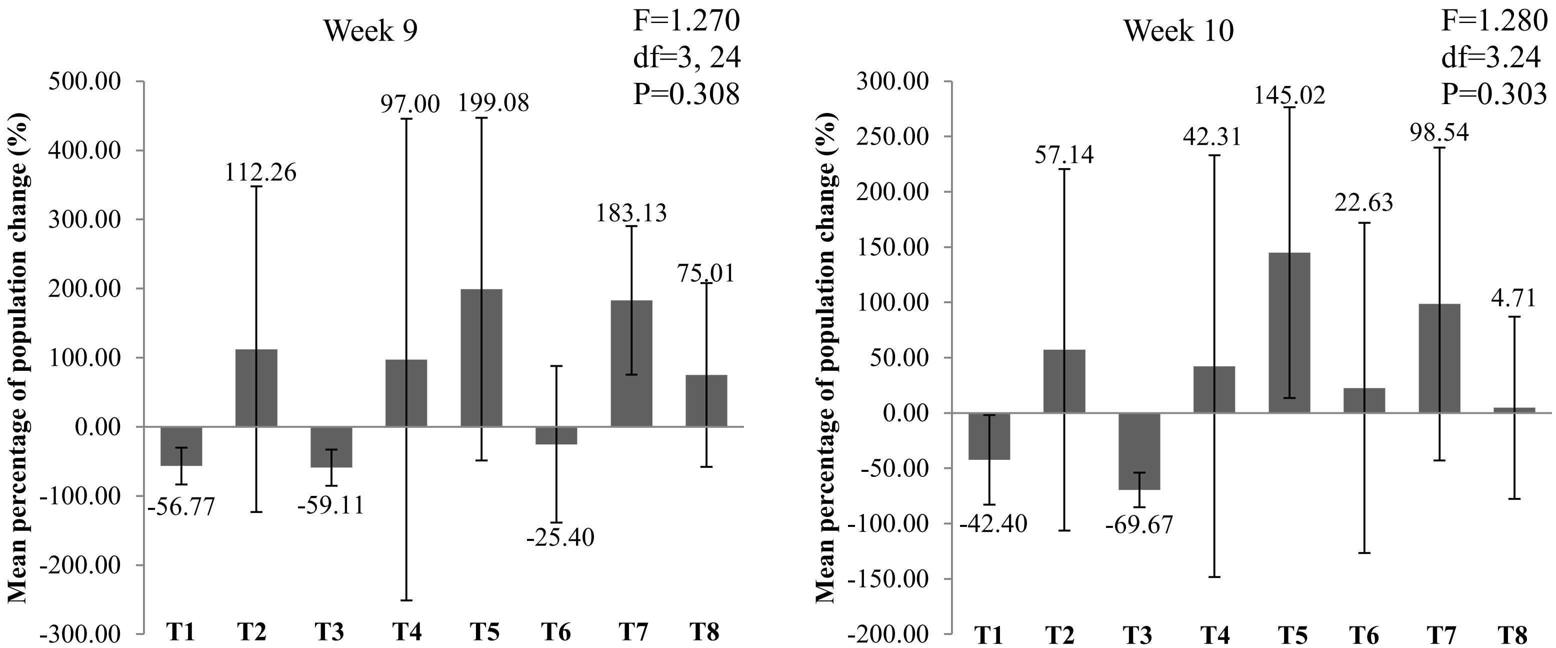

Figure 3.7 Mean (± SE) percentage *Pachycondyla chinensis* (Emery) population reduction in weeks 9 and 10 when given access to selected bait products in the field; four replicates were conducted in three locations for each treatment. The series columns from left to right are: T1-Advion® fire ant bait; T2-Advion® granule; T3-Advion® gel; T4-Advance®; T5-Optigard®; T6-Maxforce® complete; T7- Maxforce® quantum; and T8-Control.

Advion® fire ant bait showed approximately a 70% population reduction in the first three weeks. The population increased in weeks 5 and 6. After the reapplication between weeks 7 and 8, the population reduced again. Advion® granule reduced the population approximately by 65% in the first three weeks. However, the population began to increase even after reapplication of the bait after weeks 2, 4 and 7. Advion® gel showed the only reduction in population over ten weeks. Advance® was effective in the first seven weeks, and the population began to increase beginning at week 8. Optigard® reduced the population in the first three weeks when the population was also reduced at control sties. The population kept increasing after week 4. Maxforce® complete showed a population reduction in week 9 only. The population change yielded by Maxforce® quantum showed similar trend as Optigard®.

A heavy rain occurred on the afternoon after the first reapplication of broadcasted bait products. According the label, rainfall within several hours after application reduces the effectiveness of Advion® fire ant bait. The first reapplication was considered non-effective. So, the effective reapplications of Advion® granule and Advance® occurred after weeks 4 and 7, and Advion® fire ant bait was after week 7.

Discussion

1. Choice/No Choice Bait Study- Laboratory

In both choice and no-choice laboratory tests, the control groups provided with food (termite workers) and water daily, had a survival rates at 90-95%. Food also was provided in the choice test in each treatment dish.

Advion® fire ant bait, Advance® and Maxforce® complete yielded significantly higher mortality after three to four days than the other treatments. These are all granular bait products. The mortality of the granular bait, Advion® granule, was significantly lower than the other granular baits which achieved 100% mortality, and not significantly different from the control after 14 days in the choice test and 11 days in the no-choice test. The label for Advion® granule indicated that target species range widely, but attractant information was not provided. Based on observations during the 14-day test period, *P. chinensis* did not retrieve as much Advion® granule as the other three granular baits. The significantly lower mortality observed with Advion® granule may be lack of palatable attractants.

The mean percentages of *P. chinensis* mortality when exposed to gel baits, Advion® gel, Optigard® and Maxforce® quantum, were significantly different in both choice and no-choice tests. In the no-choice test, the mean mortality of Advion® gel and Optigard® were not significantly different over 14 days. However, they were significantly different when the alternate food choice (termite workers) was provided. When there was a choice between a preferred natural food source and a bait product, Optigard® was significantly less effective than Advion® gel. The active ingredient is different in these two products. The difference in the mode of action of chemicals may have resulted in variable mortality, but the significant differences seen in the choice and no-choice test indicated Advion® gel is more palatable to *P. chinensis* than other products tested. The mortality of Maxforce® quantum was significantly lower than Advion® gel. In Maxforce®

quantum, dead ants were found stuck in the bait each day. *Pachycondyla chinensis* was attracted to Maxforce® quantum but the success of bait retrieval is unknown.

Advion® fire ant bait, Advion® granule and Advion® gel contains the same active ingredient. Each chemical was provided *ad libitum*. Advion® fire ant bait killed all ants within three days when there was choice between it and a preferred natural food. An active ingredient with delayed toxic effect (<15% kill at 24h and > 89% kill at the end of test) was considered effective in controlling of the red imported fire ant (*Solenopsis invicta* Buren). Advion® fire ant bait killed approximately 40% ants within 24hours. The rapid action of this bait may affect its transport back to the larvae. Advion® gel trials did not result in significantly different mortality from Advion® fire ant bait (100%) after 11 days (85.30%) when provided with a choice, or at 7 days (84.77%) when there was no choice. Advion® granule was not significantly different from the control when ants were given a food choice. When they did not have a food choice, the mortality (21.16%) was significantly different from control (8.61%) after 11 days. The mean percentage of *P. chinensis* mortality with Advion® granule was significantly lower than with Advion® fire ant bait and Advion® gel. Since all three products contain the same active ingredient, the mode of action is the same between these treatments. Advion® fire ant bait was more effective than Advion® gel against *P. chinensis* probably due to a combination of a palatable attractant and granular formulation.

2. Field Study

The field study began in late May which was when the population was beginning to increase (Zungoli and Benson 2008). The number of *P. chinensis* collected in pitfall traps at control sites decreased in the first three weeks and increased over the remaining seven weeks of the study. Every granular bait product was observed being removed by *P. chinensis* within a five-minute period immediately after it was applied. Dead ants were observed in stations containing gel products every week.

There were many uncontrollable variables in the field study. It was difficult to schedule an application day because of higher than normal summer rainfall. The first reapplication after week 2 was affected by a non-forecasted storm. The negative influence of rainfall on broadcasting is clearly stated on the product labels. Besides the effects on chemical performance, heavy rainfall also flooded the pitfall traps which were not perfectly flushed with soil line, low lying or close to drainage. Some pitfall traps were washed out during weeks of heavy rain and resulted in sample loss.

Chemical applied by broadcasting and not removed by ants on the application day, was wetted with dew overnight. No ants were observed removing wet bait when examining the study sites on the second day. The remaining baits, Advance® and Advion® granule, were found moldy on the ground. The amount of Advion® granule applied was the highest, followed by Advance® and Advion® fire ant bait the least. The time available for ants to retrieve acceptable baits is critical to prevent baits from becoming unpalatable due to environmental factors. The amount of bait applied is economically important for practical pest management.

The results in the laboratory study showed Advion® fire ant bait, Advance® and Maxforce® complete were highly attractive to *P. chinensis* and achieved approximately 100% mortality in three to four days when there was a preferred natural food choice.

Advion® fire ant bait reduced *P. chinensis* populations in most of the study weeks in the field, and it killed *P. chinensis* faster than a preferable delayed toxic bait. As with most ponerine ants, *Pachycondyla chinensis* does not share food with other workers, but does engage in trophallaxis with larvae (Soroker et al. 1998, Rice 2012). Thus distribution of bait through the colony will rely on food being transferred to larvae. The rapid toxic effect of Advion® fire ant bait may kill a large number of workers and larvae might result in survival of non-fed ants and pupae. There might be resurgence in the population if prompt reapplication is not carried out. Warner et al. (2008) used Advion® fire ant bait for control of the bigheaded ant (*Pheidole megacephala* Fabricius) and showed similar population recovery around 20 days after exposure.

Advance® was effective in the first seven weeks and lost effectiveness by the end of the study. In the beginning of the field study, *P. chinensis* showed no significant food preference (Chapter 2). By the end of the study, a significantly different preference for protein was detected. The attractant component of Advance® is not stated in the label. Besides the possible effects by environmental factors and toxicant performance, the food attractant in Advance® may not meet the food preference shift during the test period.

Maxforce® complete was applied in stations, and ants were observed retrieving it on the second day. According to Rice (2012), the application of Maxforce® complete bait either in clumps or scattered were not significantly different in the control of *P. chinensis.* The station prevented bait from being wetting by dew, rainfall, and irrigation. However, Maxforce® complete did not show an acceptable population reduction in the field study as it did in the laboratory. Rice (2012) used Maxforce® complete against *P.*

chinensis in North Carolina (USA) providing rapid control and lasting at least 28 days with a single treatment. The evaluation of population changes was different from this study and the results were not similar. Consideration of the effects of environmental factors is critical for application of Maxforce® complete.

An excess of well-preserved bait is also attractive to non-target species. In the laboratory study, there were no competitors. But, there are many other arthropods in the field. Maxforce® complete is labeled to control ants, silverfish, crickets and cockroaches. A wide range of targeted species increases the competition. In the food preference study in the field, *P. chinensis* was observed away from the station when it was occupied by carpenter ants (*Camponotus spp.*), flies (Diptera), and field ants (*Formica spp.*) (Unpublished data). *Pachycondyla chinensis* may not compete effectively with larger-bodied arthropods. The Argentine ant (*Linepithema humile* Mayr) also was found in stations at one of the study sites. The mass recruitment of *L. humile* may reduce the foraging of *P. chinensis* for this bait when the *P. chinensis* population is small (Rice 2012). A bait product designed to attract a broad range of species may not be as effective in the field study as in the laboratory study.

There was observed competition in the field bait study from other arthropods. Pill bugs (Armadillidiidae) and millipedes (Diplopoda) often were in the stations that contained gel bait. Advion® gel which showed significantly higher mortality than other gel bait products in the laboratory study effectively reduced the *P. chinensis* population over ten weeks. Many *P. chinensis* and competing arthropods were found trapped in the stations with Maxforce® quantum ant bait. Optigard was less attractive than Advion® gel to *P. chinensis*. A proper station for gel bait with an entrance which allows only the body size of *P. chinensis* access would be an ideal design, but may not be economically efficient.

Advion® granule was removed by *P. chinensis* after application. In the first three weeks, it reduced the population up to 78%. The first three weeks of field data were dramatically different from the results in the laboratory study. The delivery form of products needs to be studied further.

Pachycondyla chinensis population increases from May to the peak time in early August. When active foraging ants are detected in the field, application of Advion gel, Advion® fire ant bait, or Advance® can be used to manage *P. chinensis*. Advion® gel was applied in bait stations and showed continued reduction in population. Advion® gel may be used to suppress a field population over a long-term period in a protected station. As hypothesized, bait including both protein and carbohydrate was more effective than bait labeled targeting sweet-feeding ants. Knowledge of the targeted area, such as the vegetation, irrigation, and surrounding arthropods, which are also in the targeted species list in the label, is also important. Irrigation may cause chemical run-off and reduce bait effectiveness. The combination of these effective baits with other general ant control methods needs to be further evaluated for a better management plan in varied urban environment.

SUMMARY AND RECOMMENDATIONS

Pachycondyla chinensis (Emery), commonly known as the Asian needle ant is an invasive ant of medical and ecological importance in the United States. The sting can cause an allergic response in humans which varies from local skin reaction to systemic reaction. *Pachycondyla chinensis* has demonstrated a strong negative impact on native ant species abundance and diversity. The ability of using termites as a food source, particularly *Reticulitermes virginicus* (Banks), was considered a springboard for their success as an invasive species and speculatively may deplete native subterranean termite populations and negatively impact long-term ecosystem processes.

Pachycondyla chinensis establishes nests around buildings and landscaping in urbanized areas in South Carolina (USA). Foraging ants are found around or under protected locations such as sidewalks, flowerbeds, mulch, tree bases, stones, and logs where human outdoor activity takes place. Colony treatment and perimeter barriers which are generally used in ant control are impractical against *P. chinensis* considering the nesting characteristic. Toxic bait applications using selected bait products currently on the market were evaluated in laboratory and field studies and are reported in this thesis.

Before the evaluation of bait effectiveness against *P. chinensis*, a food preference study was performed in both the laboratory and field. Ants were field collected and brought into the laboratory. The separation of ants from field debris resulted in a loss of larval and pupal stages. Foods were made of mixtures of agar and sucrose, vegetable oil, and canned tuna. There was no significant difference observed, during the food preference laboratory study using *P. chinensis* worker ants. All foods (carbohydrate, lipid, and protein) were visited.

A field study of food preference was conducted at the time when the *P. chinensis* population began to increase in late May and early June, as well as at their peak population in late July and early August. Four foods, carbohydrate, lipid, protein, and the food matrix (plain agar) were placed close (~20 cm) to the nest entrances to reduce the effect of foraging distance on ant food choice. The number of ants visiting each food was recorded and computed into proportions at each site in the urban environment. Multiple behaviors, such as scouting, individual food carrying, liquid removal on site, and recruitment by tandem carrying, were observed. Ants were observed to stay away from food occupied by carpenter ants (*Camponotus spp.*), flies (Diptera), and field ants (*Formica spp.*). The body sizes of these competitors were larger in comparison to *P. chinensis*. During the period of early population growth, *P. chinensis* visited all four foods, but only visited protein at a significantly higher rate than the food matrix. At the time of peak population, protein was significantly preferred over carbohydrate, lipid, and the food matrix.

Seven bait products, including formulations of four granular, two gel, and one syrup bait which is close to liquid form were chosen to evaluate the effectiveness against *P. chinensis*. In the laboratory test, bait products were provided to twenty worker ants and five pupae in each replicate. Choice between a natural food source (termite worker) and chemical was tested. A no-choice test was provided with bait only. The mortality was record for 14 days. Overall, Advion® fire ant bait, Advance, Maxforce® complete achieved 100% mortality in less than one week. Advion® gel reached around 90% mortality and was not significantly different from those reaching 100% mortality after 14 days. When there was a choice of a natural food source, Optigard® was less effective than in a no choice test that was not significantly different from Advion® gel. Optigard® was not significantly different from Maxforce® quantum in the choice test, while Maxforce® quantum achieved a mortality of 40%. Advion® granule was the least effective bait in both choice and no-choice tests and was not significantly different from control when there was a choice.

Evaluation of bait products in the field was conducted in urban areas where active foraging ants and potential nesting sites were located. Each site was identified by non-overlapping foraging resources. Pitfall traps were placed in the ground to monitor *P. chinensis* populations at each site. The population change was calculated by comparing the weekly number of *P. chinensis* collected in pitfall traps with the number collected before treatment was applied. Field results at 10 weeks showed the effective bait products differed from the effective bait products in the laboratory tests. Only Advion® gel reduced the field population over 10 weeks. Advion® fire ant bait achieved 70% reduction in the field population during the first three weeks; however the population increased during week 5 and 6. After reapplication, the population was reduced again. Advance® was effective in the first seven weeks and had no effect on the population reduction in the last three weeks even after reapplication. Maxforce® complete was not as effective as in the laboratory, and the field population increased during most weeks of the study. Optigard® and Maxforce® quantum had similar trends in population change during the 10 weeks. The population increased during weeks when there also was an increase at control sites and decreased in weeks when there was a decrease in the *P. chinensis* population at control sites.

Pachycondyla chinensis populations begin to increase from May to the peak time in early Aug. When actively foraging ants are detected in the field, application of Advion® gel, Advion® fire ant bait, or Advance® can be used to manage *P. chinensis*. Advion® gel was applied in bait stations and showed continued reduction in the population. Advion® gel may be used to suppress a field population over a long-term period in a protected station. Broadcasted bait was less palatable when they were wetted by dew. Consequently broadcasted bait applications should be made to coincide with optimal ant foraging. Knowledge of the target area, such as the vegetation, irrigation, and other arthropods listed as target species on the bait product label, also is important. Irrigation may cause chemical run-off and reduce bait effectiveness. Other arthropods in the treatment area can be competitors to *P. chinensis*. The combination of these effective baits with other general ant control methods need to be further evaluated for a better management plan in varied urban environment.

Recommendations

Pachycondyla chinensis nest under objects where moisture is stable and beneath soil surface to a depth of 3 to 10 cm. When designing a management program for *P. chinensis*, locate nests and potential nesting sites as thoroughly as possible. Reducing preferred nest sites by altering landscaping is important to prevent *P. chinensis* from establishing. Although not tested within the research conducted for this thesis, a spot treatment with residual insecticide spray applied to nest sites should provide rapid control when the population is high in the summer. *Pachycondyla chinensis* can move their nest very rapidly after a disturbance. Application equipment must be prepared in advance to optimally target a large number of ants. After activity is reduced, a proper bait product, such as Advion® gel with timely reapplication, can be used for long-term control of *P. chinensis*. Fast-acting baits, such as Advion® and Advance® can be used when there are signs of population increases. Regular inspection is necessary after treatment to time reapplication to prevent resurgence in the population. Fast-acting bait also can be used to reduce large populations at the beginning of the season. However, the amount of bait required for satisfactory control may be costly. After successful control is achieved, reducing preferred nesting habitat is still important to prevent possible immigration of *P. chinensis* from untreated area.

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